VOL. 38, PART 3 SEPTEMBER 1955

The British Mycological Society

(Recognosce notum, ignotum inspice)

TRANSACTIONS

Edited by

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CAMBRIDGE UNIVERSITY PRESS
LONDON: BENTLEY HOUSE, N.W.1
NEW YORK: 32 EAST 57TH STREET, 22

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Trans. Brit. mycol. Soc. **38** (3), 193–201 (1955).

FUSARIUM DISEASES OF GLADIOLUS

By E. W. BUXTON*

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(With Plate 5 and 1 Text-figure)

Isolates of Fusaria of the *Elegans* section cause two major types of disease in Gladiolus. One, a rot of stored corms, has long been known in Britain. The other, probably the same as the North American Fusarium Yellows (McCulloch, 1944), has only recently been recorded here, but is widespread and occurs in nearly all commercial stocks of susceptible varieties.

The two diseases have been regarded in America as etiologically distinct, corm rot being attributed to *Fusarium oxysporum* Fr. var. *gladioli* Massey, and yellows to *F. orthoceras* Woll. var. *gladioli* McCull. This seems not to be so in Britain. Fusaria isolated from rotting corms caused yellows in growing plants and isolates from yellowed plants rotted corms in storage. Whether different isolates caused yellows or corm rot, both or neither, depended on the Gladiolus cultivar to which they were inoculated. Corms that resisted invasion by some isolates formed cambial tissues and cork barriers.

The pathogenic properties and taxonomic characters of the different isolates so overlap that it seems most reasonable to classify all of them as *F. oxysporum* Fr. f. sp. *gladioli* (Massey) Snyder & Hansen.

INTRODUCTION

A Fusarium Rot of stored Gladiolus corms in America was attributed by Massey (1926) to *Fusarium oxysporum* Fr. var. *gladioli* Massey. This disease is well known in Britain and was noted by Moore (1939) occurring in imported corms. A disease of growing Gladiolus plants was recorded by McCulloch (1944), who identified the pathogen as *F. orthoceras* Woll. var. *gladioli* McCull. This disease shows by a sudden wilting and yellowing of the foliage and is widely known in the United States of America and on the continent in Europe. Both diseases also occur in Australia (Fraser, 1946), Canada (Conners, 1938), Italy (Ciferri, 1949) and the Argentine (Carrera, 1947). Field specimens were collected from Holland during the summer of 1952. Robertson (1951) recorded a disease of Gladiolus in Britain almost identical with that described by McCulloch, and this condition may be the same as that investigated by Moore and Brown (Moore, 1939).

The fungi associated with these two diseases are members of the section *Elegans* of the genus *Fusarium* Link.

The taxonomic features of isolates from Gladiolus and the variations exhibited in response to changes in cultural environment are described in the accompanying paper (Buxton, 1955).

The two diseases were studied primarily to investigate inter-relationships between the course of infection, symptomatology and host and

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Vol. 38, Part 2 was issued 21 June 1955

parasite reactions. This work was combined with a small survey of the diseases in commercial Gladioli and an evaluation of their effects on the Gladiolus industry in Britain. It was soon found that the disease pattern was obscured by variation in the Fusaria, both in culture and as pathogens, and by differences in host reaction shown by a number of Gladiolus cultivars.

This paper describes inoculation experiments which show that the relationship between diseases of Gladiolus due to *Fusarium* are more complex than hitherto described.

SYMPTOMS AND DISTRIBUTION

The symptoms of Fusarium Yellows as it occurs in Britain have already been described by Buxton & Robertson (1953). The *Fusarium* invades the young roots of growing corms at scattered points along their length, causing elongated dark brown lesions. The root crown is invaded later and the basal plate of the corm is penetrated. In some resistant varieties, e.g. Picardy, there is no further development and no above-ground symptoms appear. In all invaded roots, the *Fusarium* hyphae can be seen inside the vascular tract and inner cortex.

Storage Rot of corms due to *Fusarium* shows as dark brown concentrically ridged surface lesions, mainly at the corm base but also often at the nodes. The ground tissue of the corm becomes dry and the whole corm soon becomes black and mummified. Many corms rot because the fungus extends from the vascular tract after invading the roots during the growing season. Plants showing Fusarium Yellows symptoms, from which isolations were made, were obtained from many parts of Britain. Fusaria were isolated from thirty-three different cultivars of Gladiolus of the Colvillei, Nanus and Grandiflorus groups showing typical symptoms. Isolations were also attempted, but failed, from a further seventeen cultivars of these groups showing symptoms approximating to those of Fusarium Yellows. Primulinus types of Gladiolus were rarely found showing typical Yellows and those which were yellowed rarely yielded *Fusarium*. Specimens were obtained from localities as far apart as Ayrshire and Cornwall, but no clear-cut relationship could be found between disease incidence and soil type or climatic conditions. Magie (1953), who considered Yellows to be the most serious disease of Gladiolus in America, states that the disease is most prevalent on light sandy soil and is favoured by heavy rainfall and a warm climate. In Britain, the damage caused by *Fusarium* during the growing season varies from year to year, mainly because new stocks are frequently imported and a simple crop rotation is often practised. Although no quantitative survey has been made, about one-third of all losses from disease in growing Gladioli appears attributable to *Fusarium*, with additional high losses from Fusarium Rot of the stored corms. In Holland during the growing season (July) in 1952 Fusarium Yellows seemed to be of secondary importance to both *Sclerotinia* and *Botrytis* diseases, but as many stocks arriving in Britain from Holland have Yellows it cannot be regarded as unimportant there.

INOCULATION EXPERIMENTS

Yellows disease

During the course of the investigation, 157 different isolates of Fusaria of the section *Elegans* were retained in culture, and about twenty were selected for use in the experiments. Isolates derived from single microspores, obtained either from the first growths from the corm cores of yellowed plants taken from the field, or from surface rots of corms in storage, were transferred to tubes of dry, sterile, sieved Kettering loam and stored at laboratory temperature to provide a standard inoculum for future work (Fusaria stored in soil less readily lose wild-type characteristics). Inoculations were made in a large unheated glasshouse at the Rockefeller Field Station, Cambridge. Corms were grown, three to each 10 in. pot, in unsterile John Innes potting compost. Inoculum was prepared by growing the isolates in conical flasks containing about 300 g. of 3 % maize-meal sand, with 13 g. water per 100 g. of mixture, for 3 or 4 weeks before use. A pinch of this inoculum, previously diluted 5 times with clean sand, was placed in the soil beneath each corm at planting time. In all the experiments, interveinal yellowing of the leaves concomitant with internal corm discoloration was taken as the criterion of infection. Fusaria identical with those used as inoculum were consistently recovered from samples of corm cores and root fragments from inoculated plants when plated on 2 % potato-dextrose agar. All plated material, both from field specimens and from experimental treatments, was surface-sterilized for 1 min. in 1 % silver nitrate solution, passed through a sterilized 15 % solution of common salt and subsequently given three washes in sterile water (Davies, 1935).

Preliminary tests with seedlings, cormlets and adult flowering-size corms showed that the first two, although showing distinct symptoms, were not entirely suitable for infection studies. Inoculated seedlings had a poor emergence rate and their leaves yellowed when grown to a height of about 6 in.; inoculated cormlets also emerged poorly and showed some yellowing and both internal and external corm rotting. Adult corms were used for all subsequent work. Cross-inoculations were made in order to attempt to delimit the range of infectivity of each particular *Fusarium* isolate. Differences in degree of pathogenicity towards a particular range of Gladiolus cultivars, as judged by the production of yellowing symptoms in the growing plant, are shown in Table 1. Differential reactions of cultivars towards each individual inoculum are also evident.

Four of the seven possible combinations of infection with three cultivars are shown in Table 1: Paul Rubens alone infected (isolates 82, 90, 95); George Hommerson alone infected (isolate 104); George Hommerson and Paul Rubens infected (isolates 78, 152 R); all three cultivars infected (isolates 87, 116). Fusaria from corm rots clearly can cause typical yellowing symptoms when introduced into the soil as inoculum (isolates 116, 152 R). Isolates from cultivars other than those under test can cause yellowing within the range of susceptible plants. This has already been demonstrated (Buxton & Robertson, 1953) in inoculation tests using

Fusaria from Polynesie and Wedgwood tested against Paul Rubens, Lavender Dream, George Hommerson and Picardy cultivars. Members of each cultivar can be assumed to be genetically similar owing to their clonal popagation.

Table 1. *Cross-inoculation experiment showing number of yellowed plants due to attack by different isolates of* Fusarium oxysporum

Fusarium oxysporum			Host (no. of plants yellowed)		
Isolate No.	Origin	Cultivar	George Hommerson	Paul Rubens	Picardy
78	Corm core of yellowed plant	Tristan	4	9	0
82		Goya	0	10	1*
87		Wedgwood	12	12	5
90		Polynese	1	12	1*
95		Agitato	0	8	0
97		Lavender Dream	0	0	0
104		Paul Veronese	5	1	1*
105	Surface rot of stored corm	Polynesie	0	2	1*
152 R		Gladiolus 'colvillei'	3	8	0
152 W		Gladiolus 'colvillei'	1	3	0
116		George Hommerson	10	11	6
Controls			0	0	0
No. of isolates causing yellowing in each cultivar			5	8	2

* Cases in which yellowing was atypical and shown to be due to other causes.

The figures show the number of plants yellowed (interveinally) out of twelve inoculated in each set.

The ability of these Gladiolus Fusaria to infect other hosts known to be susceptible to attack by closely related (morphologically indistinguishable) members of the section *Elegans* was tested. Susceptible varieties of pea, aster, flax, cotton and freesia were inoculated by the same method as used in Gladiolus tests, using mixed inoculum of isolates 90 and 87. The first four remained uninfected, but typical yellowing symptoms, together with root damage and internal corm rotting, occurred in freesia (Pl. 5, figs. 1, 2 *a*, *b*), which is a member of the Iridaceae closely related to Gladiolus. Gladiolus cultivars known to be susceptible to Fusarium Yellows were inoculated with pathogenic isolates of *Fusarium oxysporum* from wilted aster, flax, water-melon and potato, but none became diseased. These tests indicate the strict pathogenic specificity of culturally indistinguishable members of the *Elegans* section of *Fusarium*.

Corm-rotting phase

Isolates of *Fusarium oxysporum* were obtained from lesions of the type described by Massey (1926) occurring on corms newly imported from abroad and from stocks established in this country. Having demonstrated (Table 1) that such isolates, together with those from corm cores of yellowed plants, can cause typical Fusarium Yellows symptoms, samples of Fusaria from both types of disease were tested for their ability to cause rotting of stored corms. Two methods of investigation were adopted. The first was to search for rotting in stored corms from plants used in the growing season inoculation experiments. The second was to inoculate clean corms and then store at 25° C. in the laboratory.

Inoculated plants which had been scored for yellowing symptoms were lifted during September, the corms were left to dry on open trays and their outer husks and parent corms removed 2 weeks later. They were then stored on trays in an unheated glasshouse and examined frequently. Signs of rotting began to appear within 6 weeks of lifting. The symptoms are fairly clear (Pl. 5, fig. 3) and, as would be expected from the previous observations of diseased corms of yellowed plants (Pl. 5, fig. 2*b*) in which the radiating vascular bundles were infected, they occurred primarily at the nodes of the corms. The lower nodes first showed symptoms; small brown areas rapidly expanded and became darker, tending almost to black, and developing characteristic concentric ridges. Examination of the interior ground tissue of the rotted regions showed that each ridge corresponded with a darkened advancement zone below the surface. This apparently cyclic tissue reaction could not be attributed to any diurnal factor, such as light or temperature fluctuations, because corms kept in the dark at a constant temperature showed similar ridged lesions.

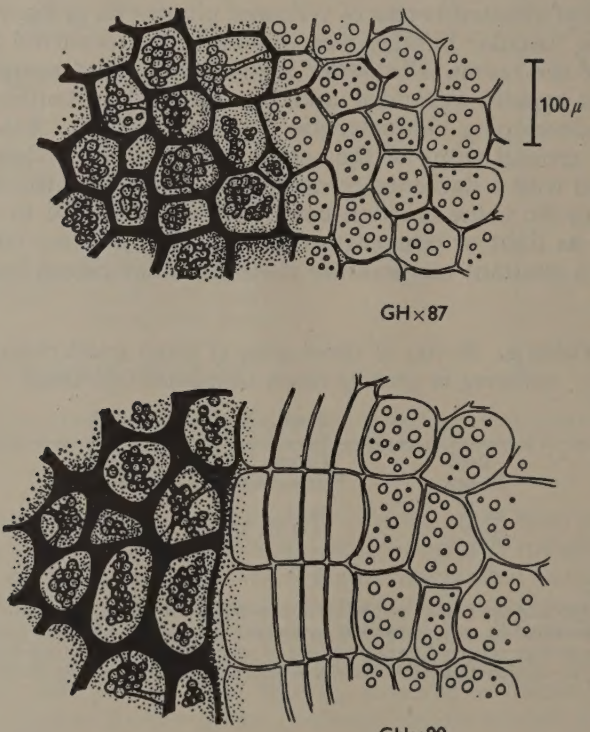
Table 2. *Rotting of stored corms of plants which showed yellowing in growing season inoculation experiments*

Original Fusarium inoculum	Host (no. of corms rotted)			
	Paul Rubens	George Hommerson	Lavender Dream	Picardy
87	20 N	15 D	30 D	15 B, N
90	20 N	11 B, D, N	4 B, N	0
116	9 B, N	15 D	0	0
Controls	0	7 D	0	0

The figures represent the pooled results of three comparable experiments, thirty corms of each cultivar being retained in each treatment by isolates 90 and 87, fifteen in each treatment by isolate 116. B=rotting occurred mostly at base of corm; D=almost complete rapid dry decay of whole corm, in conjunction with *Penicillium* spp.; N=rotting mostly at nodes (see Pl. 5, fig. 3).

Table 2 shows that three types of symptom can generally be recognized within the main corm-rotting complex caused by *Fusarium*. The numbers of plants which yellowed in each category differed (Table 1), so that Table 2 does not show any further evidence of differential susceptibility between cultivars. Picardy appeared to be resistant both to yellowing and rotting, whereas Lavender Dream corms were not rotted by isolate 116, despite the susceptibility of this cultivar to yellowing and the fact that isolate 116 originated from a corm rot (of George Hommerson).

To determine the relative abilities of isolates 90 and 87 to cause rotting, clean Paul Rubens, George Hommerson and Picardy corms were inoculated with each of the isolates. The corms were dehusked, washed in 50 % alcohol for 3 min., transferred to 1/1000 mercuric chloride for 15 min. and then washed 5 times in sterile water. After drying, small flaps of tissue, about 3 mm. deep, were cut in the sides and inoculum (young mycelium growing on 2 % potato-dextrose agar medium) was placed beneath these. Corms were then put in cardboard boxes of moistened peat and incubated at 25° C. The experiment was twice repeated, involving altogether 26 corms in each treatment. The results were recorded after 28 days and the same pattern (Pl. 5, fig. 5) was observed each time. The first experiment was in November, the last in late February, hence the physiological state of the dormant corms had no effect on the infection. Pl. 5, fig. 5, shows that the Picardy corms resisted attack by isolate 90, whereas George Hommerson and Paul Rubens corms showed consistently different degrees of infection. There is a clear distinction on Picardy corms between these two physiologic strains of Gladiolus *Fusarium*.



Text-fig. 1. Sections of corm tissue of cultivar George Hommerson showing differences in reaction to infection by two strains of the Gladiolus *Fusarium*. A cork cambium is clearly visible in corms attacked by isolate 90.

The difference between the reactions to isolates 90 and 87 shown by any one corm cultivar appeared to lie in their ability to form a cork cambium across the path of the invading fungus. Sections across the junction of clean and diseased tissue in rotting corms consistently showed the cork cambium.

No cambial activity could be demonstrated in corms invaded by isolate 87, in which gum and tannin deposits, together with thickening of the cell walls and breakdown of the invading hyphae, occurred as a gradation from the healthy to the diseased tissue. Since these details were observed, they have been independently confirmed by Bald (1953), who also states that the cambial activity is a function of the storage temperature.

ETIOLOGY

The inoculation experiments showed that *Fusarium* invades the corms through the roots and corm bases. Quite early in the growing season, the lower parts of the vascular tract of the new corms become discoloured and *Fusarium* can be readily isolated from such regions. At lifting-time, infected plants have corms which show discoloured vascular tracts radiating from the central core. Such corms, when stored, soon develop lesions at the nodes. The evidence presented above indicates that the two conditions hitherto known as Fusarium Yellows and Storage Rot and thought to be etiologically distinct can have the same cause. Once the *Fusarium* has become established in a stock of corms by infection from the soil, eradication is almost impossible. Corms with latent infections which do not show as nodal rots in storage are dispersed among the stock. Roguing of stocks in storage will remove most infected corms, but removing plants which show yellowing during the growing season is also advisable.

The leaves turn yellow just before the vascular core of the corm becomes invaded by the *Fusarium*. The interveinal areas, which are the first to turn yellow, possess chloroplasts which are burst and become devoid of chlorophyll. As the *Fusarium* cannot be isolated from any part of the leaves, toxins formed in the roots or corm bases may disrupt the chloroplasts. An experiment was made to test this hypothesis. Liquid potato-dextrose medium was inoculated with pathogenic isolates of the *Fusarium* and incubated until growth ceased. The liquid was passed through a Seitz filter, and healthy leaves of Gladiolus were placed with their lower ends in the filtrate, with an equal number in uninoculated potato-dextrose liquid. After 10 days in the glasshouse, the filtrate-treated leaves turned yellow, whereas the controls did not. True wilting does not always accompany the leaf yellowing and it is probable that, in Fusarium Yellows, the leaf symptoms are caused by a combined effect of root decay and toxin produced by the *Fusarium*.

DISCUSSION

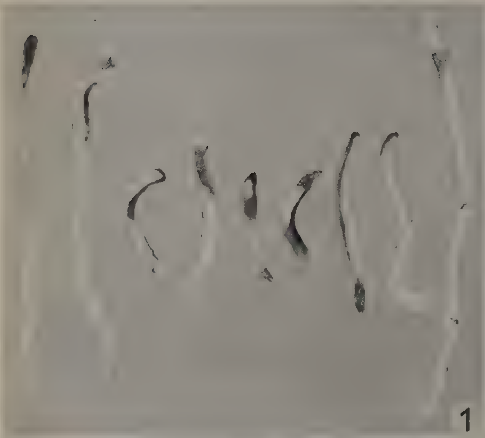
The earlier separation of Gladiolus diseases caused by *Fusarium* into two distinct disease patterns with different etiologies oversimplifies the problem, at least as it occurs in Britain. The original descriptions of the two phases of the disease complex included some confusion concerning taxonomic designation of the causal fungi. Organisms corresponding to the two Fusaria described by Massey (1926) and McCulloch (1944) as *Fusarium oxysporum* Fr. var. *gladioli* Massey and *F. orthoceras* Woll. var. *gladioli* McCulloch, respectively, have been shown to possess many overlapping pathogenic characteristics. The evidence presented in this paper (and by Buxton, 1955) suggests that it would be simpler to regard isolates of *F. oxysporum* pathogenic to Gladiolus as strains of *F. oxysporum* Fr. f. sp.

gladioli (Massey) Snyder & Hansen, proposed by Snyder & Hansen (1940) as synonymous with Massey's *F. oxysporum* var. *gladioli*. The inclusion of *F. orthoceras* var. *gladioli* in Snyder & Hansen's form species is justifiable because of the pathogenic range shown by British isolates from Gladiolus exhibiting Yellows.

Isolates from different Gladiolus cultivars retain their specificity within certain limits. Throughout the history of commercial Gladiolus growing, each particular *Fusarium* strain has become associated with its particular range of cultivars. The complex pattern of relationship of particular *Fusarium* isolates to certain Gladiolus cultivars raises the problem of the possible modes of origin and survival of such potential variability. A micro-evolution of the *Fusarium* has probably proceeded in parallel with the production of new cultivars raised by crossing and selected for aesthetic appeal. The production by mutation or the segregation of already existing specific pathogenic strains of the *Fusarium* could have enabled the fungus to follow the progression of new Gladiolus cultivars. Do the particular strains of *Fusarium oxysporum* exist in the soil as ready-made specifically pathogenic types which can only express their range when a particular cultivar is planted in their vicinity? The alternative postulate of a rapid mutation rate tending towards specific pathogenicity in the rhizosphere of particular cultivars seems less probable. There is no evidence to suggest that root exudates from particular host plants are specifically mutagenic to soil Fusaria. This important question of the mode of co-existence of the various *pathogenic* strains in the soil has so far received little attention. There certainly exists sufficient evidence to suggest that most of the potentially pathogenic strains within the macro-species *Fusarium oxysporum* (sensu Snyder & Hansen) are already present in some form in many soils. Whether or not they can fluctuate between a saprophytic and specifically parasitic existence, or occur as diminishing components of a genetic complex of *Fusarium* in the soil, is still an open question.

One interesting practical point raised by the work reported here concerns the validity of the basis for attempted control of the corm-rotting phase by dipping treatments. It has not been demonstrated here that surface lesions cannot occur by penetration of the *Fusarium* from outside through the surrounding corm husk, but it has been shown that surface lesions are often formed by the growth of the fungus through the root into the root plate and thence by way of the vascular bundles to the corm surface. When this happens there is no reason why any surface treatment of the corms should control the formation of lesions.

My thanks are due to the Agricultural Research Council for a Research Studentship during the tenure of which this work was carried out, also to Dr N. F. Robertson for supervision, and to members of the National Agricultural Advisory Service and others who kindly provided material for examination.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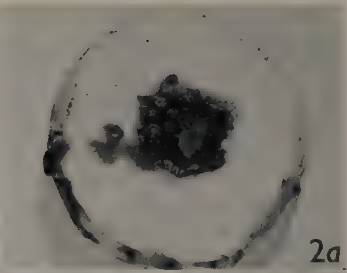
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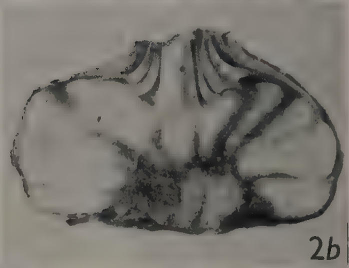
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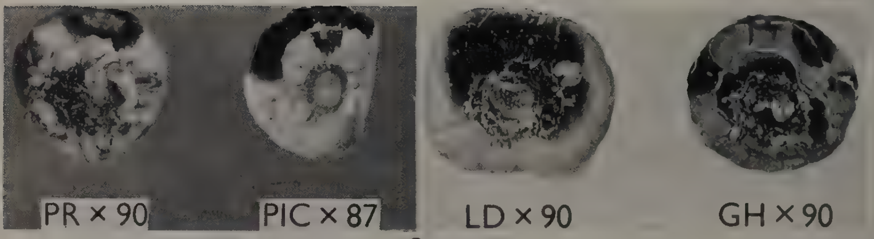

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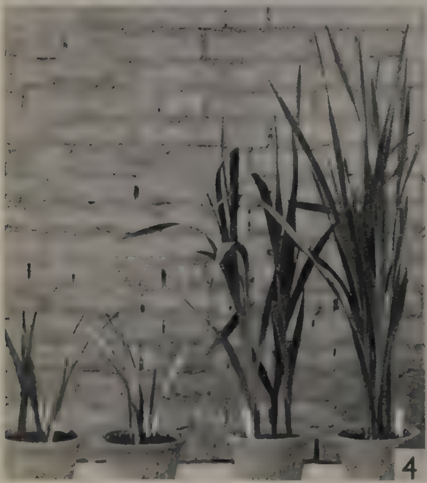
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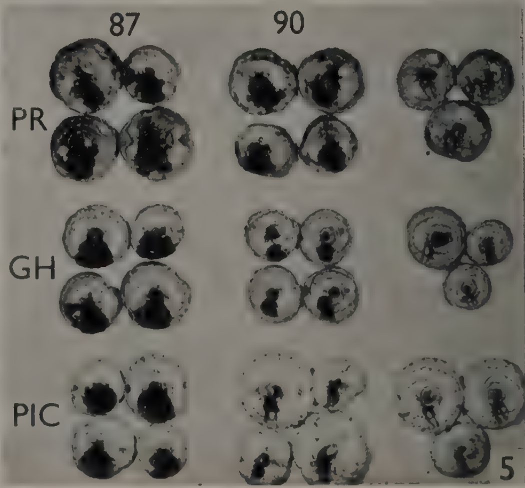

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EXPLANATION OF PLATE 5

Fig. 1. Roots of infected Gladiolus cultivar High Life (from inoculation experiments) showing attack by *Fusarium*. (× $1\frac{4}{5}$.)

Fig. 2. Sectioned corms of infected Gladiolus plants (from inoculation experiments) showing vascular discoloration caused by entry of *Fusarium* via basal plate and root cores: (*a*) Cross-section just above basal plate. (*b*) Vertical section. (× $1\frac{1}{5}$.)

Fig. 3. Nodal rotting of corms variety Paul Rubens (PR), Picardy (PIC), Lavender Dream (LD) and George Hommerson (GH) which had previously shown yellowing. (× $\frac{3}{5}$.)

Fig. 4. Gladiolus plants, cultivar Lavender Dream, (left) inoculated by isolate 90 and (right) control. (× $\frac{3}{50}$.)

Fig. 5. Corms of three Gladiolus cultivars (Paul Rubens = PR, George Hommerson = GH, and Picardy = PIC) inoculated with two *Fusarium* isolates, 87 and 90, taken from an experiment showing strain differences in the *Fusarium*. (× $\frac{1}{5}$.) Controls on right.

(*Accepted for publication* 24 *October* 1954)

Trans. Brit. mycol. Soc. **38** (3), 202–212 (1955).

THE TAXONOMY AND VARIATION IN CULTURE OF *FUSARIUM OXYSPORUM* FROM GLADIOLUS

By E. W. BUXTON*

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(With Plate 6 and 5 Text-figures)

Variations in light intensity, nitrogen concentration and pH of the culture medium affected the morphological features of isolates of Fusaria of the *Elegans* section from diseased Gladiolus to such an extent that they could not be used as reliable taxonomic criteria. The wide range of morphological expression in culture indicated that the British isolates included the taxonomic descriptions hitherto given for Fusaria from diseased plants showing premature yellowing of the foliage and storage rot of the corms. The changes in spore shape, size and mode of presentation, colony colour and type of aerial mycelium, were sufficiently wide to necessitate placing all the isolates in the macro-species *Fusarium oxysporum* Fr. sensu Snyder & Hansen. It is suggested that such Fusaria causing premature yellowing and storage rot of corms be classified as *F. oxysporum* Fr. f. sp. *gladioli* (Massey) Snyder & Hansen.

INTRODUCTION

The two major diseases caused in Gladiolus by *Fusarium oxysporum* Fr. f. sp. *gladioli* (Massey) Snyder & Hansen, Fusarium Yellows and Fusarium Storage Rot, are described by Buxton (1955). While investigating the disease, over 150 separate isolates were retained in culture and samples examined for their taxonomic relationships and pathogenicity. The isolates were made from mass hyphal tips taken from 3- to 4-day-old colonies growing from fragments of diseased roots or corms of yellowed plants, and from surface rots of stored corms on dishes of 2 % potato-dextrose agar medium (200 g./l. potato extract). Mass hyphal and single-spore inocula were transferred to tubes of sterile-sieved Kettering loam soil kept at room temperature, for use as inoculum.

A wide range of morphological variation was soon apparent in culture, and subculturing either by bulk inoculum or from single spores increased the variability. A critical examination of sample isolates showed, however, that all fell within the taxonomic limits of the *Elegans* section of the Fusaria as described by Wollenweber & Reinking (1935). This conclusion was based mainly on the shape and size of macroconidia and their mode of production, together with other differences involving considerations of colony colour, growth rate, amount of aerial mycelium and presence or absence of sclerotia. The Fusarium Yellows disease was ascribed by McCulloch (1944) to *Fusarium orthoceras* Woll. var. *gladioli* McCull., a member of the Orthocera subgroup of the section *Elegans*. The Storage Rot disease fungus was described by Massey (1926) as *F. oxysporum* Fr. var. *gladioli* Massey, a member of the Oxysporum subgroup of the

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* Now at Rothamsted Experimental Station, Harpenden, Herts.

same section of the Fusaria. Buxton (1954) showed that heterocaryons can be made between forms which can be allotted to *F. orthoceras* and *F. oxysporum* on Wollenweber's taxonomic criteria.

The genus *Fusarium* includes, according to Wollenweber, Sherbakoff, Reinking, Johann & Bailey (1925) 'all Hyphomycetes and conidial stages of Ascomycetes that have no black or pure grey colour either in the mycelium or in conidia and that have macroconidia that are acrogenous, typically septate, sickle-shaped and not round at the ends. Microconidia, chlamydospores, and sclerotia may be present'. The section *Elegans* has no known perfect stage. It is split into three subsections, Orthocera, Constrictum and Oxysporum, by Wollenweber & Reinking (1935), mainly on the basis of macroconidial shape and mode of production of spores.

Many variants of Fusarium isolates occur in culture, and their taxonomic definition presents unusual difficulties. In the section *Elegans*, this resulted in the merging of the Orthocera and Oxysporum subgroups, hence the pathogens described by McCulloch and Massey are both members of the 'macro-species' *Fusarium oxysporum* Fr. emend. Snyder & Hansen. This paper is an attempt to show how far variation in cultural conditions can affect the range of morphological expression shown within isolates of *Fusarium oxysporum* from diseased Gladiolus.

METHODS

From the collection of isolates of *Fusarium oxysporum* from diseased Gladiolus, a random selection of about fifty were examined in detail to determine the extent of variation in taxonomic features when grown on different media. A comprehensive system of recording spore sizes, shapes, mode of production, etc., under various cultural conditions was adopted, making use of annotated record sheets.

Spore measurements

In Table 1 measurements of the various features, derived by taking the limiting cases from cultures grown on a wide range of media, are compared with data given by Massey and McCulloch.

These comparisons show that the British isolates cannot be distinguished in culture from the American, and that there are all degrees of variation between forms corresponding to *F. orthoceras* as described by McCulloch (1944) and *F. oxysporum* as reported by Massey (1926). The taxonomic features of the British isolates are shown in Text-fig. 1.

An example of the variability in spore length from a single isolate grown in a specified cultural environment is given in Text-fig. 2.

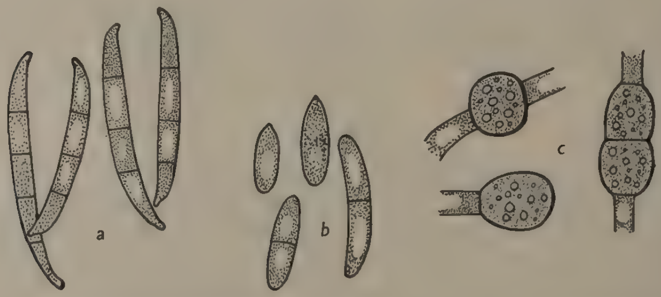
Variation on different culture media

An example of the extreme variation in colour, extent of aerial mycelium, frequency of spore types and sclerotia, shown by one isolate (no. 90) on ten different media is given in Table 2. Similar results were obtained with several other isolates on the same media.

Table 1. *Comparison of taxonomic features of the Gladiolus Fusaria by three different observers. Measurments in µ.*

Taxonomic features	McCulloch	Massey	Own observations*
Microconidia			
0-septate	3–14 × 2·2–5	Not stated	3–13·3 × 2·4–5·2
Macroconidia			
3-septate	17–55 × 2·5–5·6	25–41 × 3·5–4·8	21–53 × 2·5–6·3
4-septate	30–53 × 3·5–5·6	33–43 × 3·8–4·8	25–53 × 4·8–6·0
5-septate	26–54 × 4·2–5·6	42–46 × 4·3–4·6	25–56 × 4·5–6·0
Sporodochia	None	On cornmeal and oat agars	In some cultures (spasmodic)
Pionnotes	Very rare	Not stated	Uncommon
Sclerotia	Occasional	Fairly common	Frequent
Chlamydospores	Abundant	Abundant	Abundant
1-celled	7–10	6–14 × 5–10	6–12
2-celled	12–15	Not stated	11–15

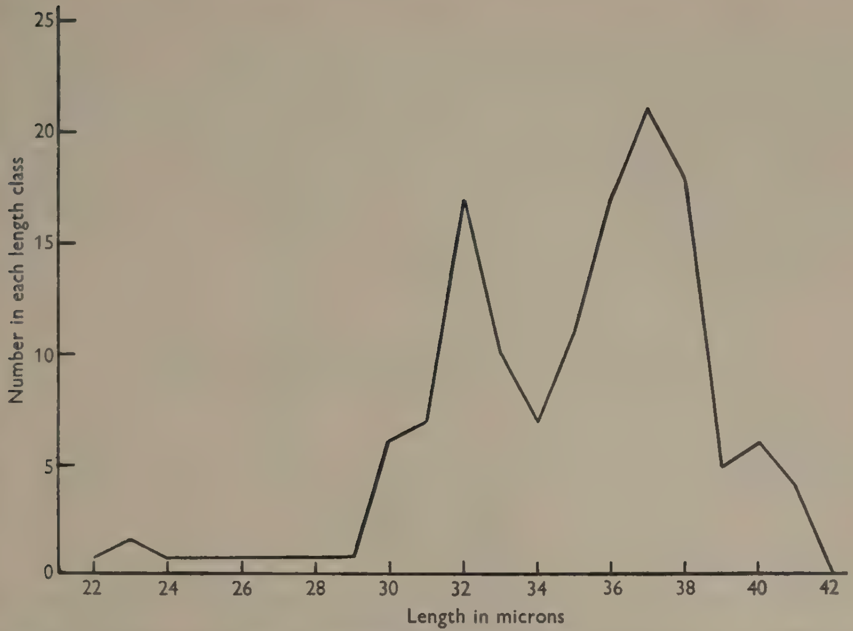
* From a count of 150 spores of each type taken from 20-day-old cultures at 25° C.



Text-fig. 1. Taxonomic features of *Fusarium oxysporum* Fr. f. sp. *gladioli* ex British material. (*a*) Macroconidia, (*b*) microconidia, (*c*) chlamydospores.

Effects of light on cultural features

Harter (1939), working with three species of *Fusarium* (not *F. oxysporum*), showed that growing cultures in the light increased the production of the macroconidia, especially as pionnotes (slime spore masses extending over the surface of the colony); and Snyder & Hansen (1941), working with other species of *Fusarium*, showed that cultures raised in light and in darkness differed greatly in their morphology. Using Gladiolus *Fusarium* isolate no. 87, raised on slants of 2 % potato-dextrose agar medium, cultures grown in light and darkness were compared. Twenty single-spore colonies of common origin were raised. Ten of the colonies, chosen at random, were covered separately with black opaque paper. All twenty were placed beneath a fluorescent lamp at 50 cm. distance. The results were recorded after 20 days and are shown in Table 3 and Pl. 6. Half of the dark set were uncovered after 20 days and left for a further 12 days. No change occurred in their morphology, indicating that the limit of time in darkness necessary to stabilize the morphology of the isolate was at the most 20 days. The next experiment demonstrates the



Text-fig. 2. Variation in length of 3-septate macroconidia from a culture (no. 75) of *Fusarium oxysporum* f. sp. *gladioli* grown for 20 days in darkness at 25° C. on 2% potato-dextrose agar.

Table 2. *Growth form, colour and incidence of taxonomic features of isolate* 90 *on various media. Cultures retained* 3 *months for observation at* 25° C. *in darkness*

Medium	Conidia	Chlamydospores	Sclerotia	Mycelium	Colour
2% malt agar	Mic. only Mac. rare	Abundant Smooth	Frequent, 0·2 mm. diam.	Woolly, some flat	White and pinkish
Czapek-Dox	Mic. only Mac. rare	Abundant Smooth	Nil	Woolly, very high	Rose and bluish
Bean stems	Mic. freq. Mac. rare	Very rare	Nil	Woolly and twisted	All white
Oatmeal agar	Mic. abundant Mac. freq.	Common Smooth	Nil	Woolly and abundant, adpressed	White and pinkish tinges
Potato-dextrose agar	Mic. freq. Mac. freq.	Common Smooth	Spasmodic, 0·2 mm. diam., dark blue	Woolly, some flat	Rocellin purple
Potato plug	Mic. freq. Mac. freq.	Very rare	Nil	Slightly cottony	White
Clover stems	Mic. freq. Mac. rare	Very rare	Nil	Cottony	Light brown
Boiled rice	Mic. freq. Mac. rare	Rare	Nil	Slightly cottony	Pale pink
Malt plus 4% glucose	Mic. freq. Mac. freq.	Frequent	Nil	Woolly	White
Dox plus yeast	Mic. freq. Mac. freq.	Frequent	Nil	Woolly	White

Mac. = macroconidia; Mic. = microconidia; Freq. = frequent.

critical length of exposure necessary to bring about pionnotal formation. Twenty slant cultures of the same isolate were exposed to the lamp. Pairs of the tubes were covered after each successive day up to 10 days, the first pair having been exposed for 1 day, the second for 2 days, and so on. The results, which are given in Table 4, show that the critical period of exposure to continuous light for colour formation is 5 days, and for pionnotal formation 3–4 days. No sclerotia occurred in any of the cultures. Apparently the exposure time necessary to form sclerotia was not reached and must, from a consideration of the data in Table 3, lie between 10 and 20 days for this particular isolate.

Table 3. *Effects of continuous light* (*fluorescent lamp*) *on* Fusarium *isolate no.* 87 *after* 20 *days' exposure*

Colour	Aerial mycelium	Spores	Pionnotes	Sclerotia
Dark set				
Deep vinaceous purple	Sparse, cottony, 0·4 cm. high	Macro. rare, straight. Micro. abundant. Chlamydospores common, spherical	Absent	Absent
Lighted set				
Orange	Very sparse, 0·2 cm. high	Macro. abundant, curved. Chlamydospores very rare, oval. Micro. rare	Abundant (Text-fig. 4)	Common, 0·5–1·0 mm. diameter. Dark blue

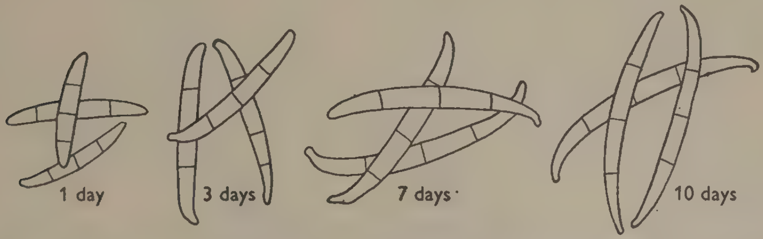
Table 4. *Effect of length of lighting on* Fusarium *isolate no.* 87

No. of days exposed	Colour	Pionnotes
1	Deep vinaceous purple	Nil
2	Deep vinaceous purple	Nil
3	Pale vinaceous purple	Very light
4	Pale vinaceous purple	Slight, brown
5	Pinkish orange	Abundant, orange
6	Pale orange	Abundant, orange
7	Orange	Abundant, orange
8	Deep orange	Abundant, orange
9	Deep orange	Abundant, orange
10	Deep orange	Abundant, orange

A further small experiment was made to test the effect of light of different colours. Duplicate slant cultures were placed, immediately after inoculation, beneath coloured glass shades of red and blue. The shades consisted of bell jars having hollow walls which were filled with potassium permanganate and copper sulphate solutions, respectively. The jars were placed in direct daylight in the laboratory window and left for 15 days. Under blue light, the cultures became orange and developed abundant macroconidia as pionnotes, whereas red light produced a pale vinaceous colour, with no pionnotes and macroconidia rare. Sclerotia were not present in either set.

Cultures of *F. coeruleum* and *F. culmorum* were grown in light and darkness without noting any differences.

For taxonomic purposes cultures of *F. oxysporum* must obviously be kept under standard lighting conditions. Perhaps the most important taxonomic criterion, that of macroconidial shape, appears to be the most likely to vary when periods of exposure to light differ. Text-fig. 3 shows the shapes and mean lengths of macroconidia of single-spore colonies of common origin which have been exposed to light for different periods.

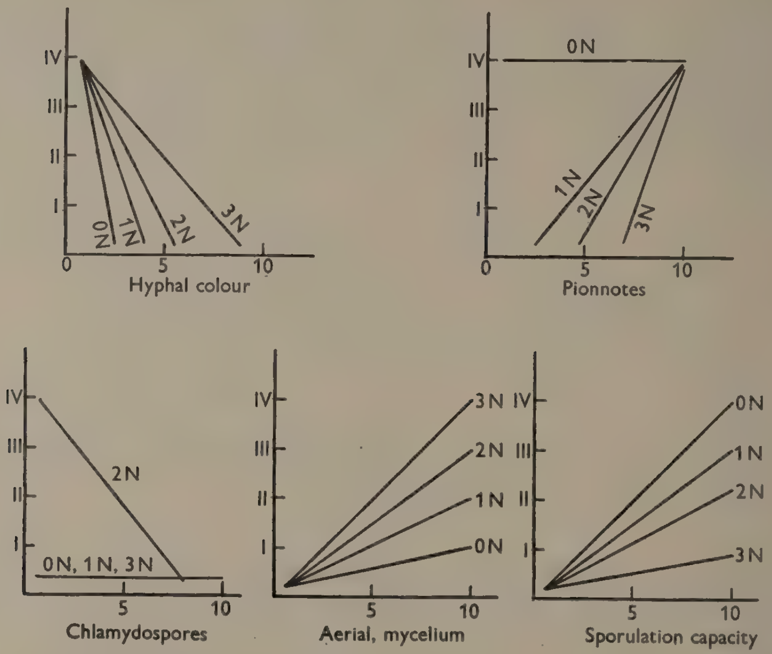


Text-fig. 3. Variation in macroconidial morphology and measurements after the colonies were exposed to different periods of lighting. Mean lengths of the conidia were: 1 day, 25 μ; 3 days, 28 μ; 7 days, 36 μ; 10 days, 45 μ.

Interaction of light and changes in the peptone content of the culture medium

Four agar media varying in their nitrogen (peptone) content were prepared. Peptone concentrations were: 0, 1·5, 3·0 and 4·5 g. per l., which are equivalent to 0, 0·2, 0·4 and 0·6 % of nitrogen, respectively, 0N, 1N, 2N and 3N representing the increasing nitrogen concentrations. The basic medium was composed as follows: glucose, 19 g.; $MgSO_4 . 7H_2O$, 0·5 g.; K_2HPO_4, 1·0 mg.; $FeCl_3$, trace; agar, 20 g.; distilled water, 1000 ml.

In this experiment each set of tube cultures was covered with opaque paper in turn each day, as described above, for 10 days. The combined effects of exposure time and peptone concentration on taxonomic features of the culture medium are shown in Text-fig. 4. The various features were estimated carefully by eye and are presented as a scale of I to IV. The variation in sporulation was estimated by haemocytometer counts and by observations of turbidity of spore suspensions. The intensity of red colour diminished with the increasing length of lighting time, but at a slower rate when the peptone content of the medium was increased. Formation of pionnotes increased with the extension of the light period, as before, but increased peptone tended to suppress this effect of light. The frequency of chlamydospores increased at 0·4 % N only, and extended lighting lessened their number. It may be that chlamydospore frequency is not a function of light or of peptone concentration of the medium. Aerial mycelium increased in amount both with extension of exposure to light and with increased nitrogen. In conjunction with this, as might be expected, sporulation capacity decreased as the peptone content increased, although lighting extension by itself favoured increased sporing. One of the non-nitrogenous constituents of peptone may be the active factor, but this possibility has not been studied.



Text-fig. 4. Trends in the variations in taxonomic features of *Fusarium* isolate no. 87 with interacting light and nitrogen content of the culture medium. Abscissa: days exposed. Ordinate: rating scale I–IV.

Influence of pH *of the medium on morphology*

The pH of the medium increased when the fungus grew on the four peptone media containing higher nitrogen concentrations. To study how pH affected morphological features of taxonomic importance, seven sets of potato-dextrose agar media (2 % dextrose, 2 % agar) were prepared, and their pH adjusted to different levels with 1/N-HCl or 1/N-NaOH.

A universal indicator was used to measure the pH of the media after autoclaving. Three isolates (nos. 81, 87 and 90) were inoculated, using disks of cultures cut with a 2 mm. cork-borer, in duplicate on dishes of each of the seven media. The cultures were incubated for 7 days at 25° C. in darkness and their morphological features were then recorded (Table 5). Exposure to light for a further 7 days did not affect their morphology. The results clearly show that the pH of the medium significantly affects taxonomic characters, both of separate isolates and within one isolate.

Increasing pH also changes colour from vinaceous purple through pink and red to pale blue. Most aerial mycelium was produced in the middle pH range. The amount of growth, measured as increase in diameter, varied considerably, while the form, measurements and relative abundance of spore types are also clearly functions of the pH.

Table 5. *Changes in taxonomic features of three Gladiolus* Fusarium *isolates when grown on a range of media varying only in* pH

COLOUR

pH	Isolate 90	Isolate 87	Isolate 81
9·1	Pale indigo blue	Very pale blue	White
8·6	Light vinaceous	Pale red-bluish	Pale yellow
8·2	Light purple	Pale spinel red	Pale buff
7·0	Etruscan red	Medium purple	Pale buff
6·5	Deep vinaceous	Dark spinel red	Deep rose pink
4·0	Dark spinel red	Very deep red	Light purple
3·3	Vinaceous purple	Pale rose pink	Light purple

GROWTH FORM—AERIAL MYCELIUM (figures show height in mm.)

pH	Isolate 90	Isolate 87	Isolate 81
9·1	Coarse, woolly 1	Coarse, woolly 1	Woolly, twisted 5
8·6	Coarse, woolly 2	Adpressed 0	Woolly, twisted 6
8·2	Coarse, woolly 2	Adpressed 0	Woolly, twisted 6
7·0	Very sparse, 0·5	Woolly, white 2	Woolly, twisted 6
6·5	Woolly, red 2·5	Woolly, red 3	Pink, cottony 5
4·0	Woolly, red 3	Woolly, red 3·5	Pink, cottony 4
3·3	Woolly, red 1	Woolly, pink 1	Pink, cottony 2

GROWTH RATE (as diameter in cm. after 7 days)

	pH						
No.	9·1	8·6	8·2	7·0	6·5	4·0	3·3
90	9·0	8·5	8·0	7·3	4·0	1·8	0·8
87	8·6	3·2	3·0	3·0	3·0	2·0	0·7
81	8·5	9·0	9·0	8·0	4·2	1·6	1·0

CONIDIA

No.	pH 9·1	pH 3·3
90	Mac. absent, Mic. very abundant	Mac. absent, Mic. very rare
87	Mac. absent, Mic. abundant, 2-celled	Mac. very rare, Mic. frequent
81	Both types abundant, curved	Both types rare, quite straight

CHLAMYDOSPORES

No.	pH 9·1	pH 3·3
90	Common, intercalary and terminal	Abundant, intercalary and terminal
87	Rare, intercalary only	Abundant, mostly intercalary, rarely terminal
81	Absent	Abundant, intercalary and terminal

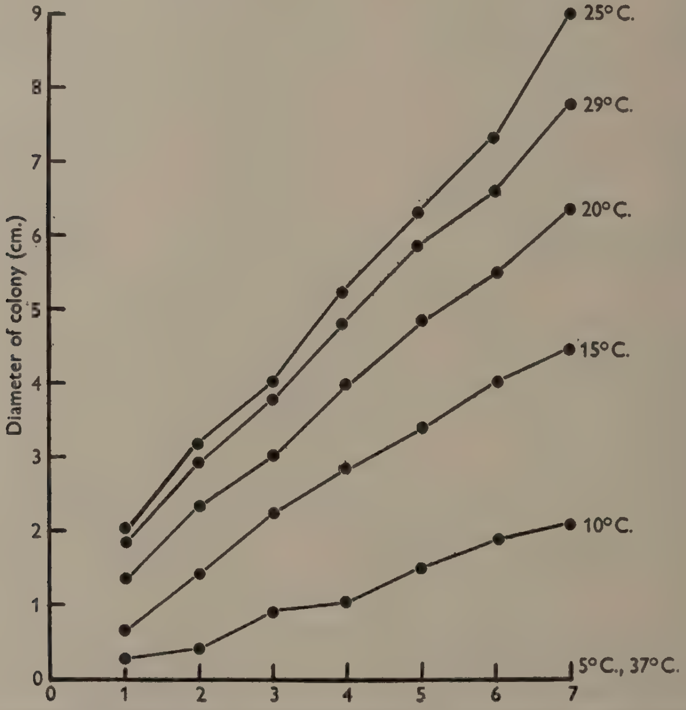
HYPHAL FORM

No.	pH 9·1	pH 3·3
90	Straight, thin, spreading, smooth. Oil droplets present	Thick, stunted, knobbly, matted and stiff. Abundant oil
87	Smooth, thin. Few oil droplets	Gnarled, lumpy, stiff and stunted at margin. Abundant oil
81	Straight, thin, smooth. Oil droplets rare	Lumpy, stiff, matted. Oil droplets common

Growth rate and temperature

Having shown that growth rate depends on the type of culture medium, the effect of temperature was next investigated. Single-spore cultures of isolate 90, of common single-spore origin, were inoculated in triplicate at the centre of dishes of 2 % malt-extract agar, and incubated in constant-temperature rooms, at the Low Temperature Research Station, Cambridge, kept at eight different temperatures varying from 0 to 37° C. The successive changes in colony diameter are plotted in Text-fig. 5.

There was no growth at 0, 5 and 37° C.; the optimum temperature for growth was *c.* 25° C. Most of the other members of *F. oxysporum* which have been investigated have the same general range and optimum temperature requirements. Most aerial mycelium developed at the lower temperatures and the mycelium was a deeper red at the higher temperatures.



Text-fig. 5. Growth rate of *Fusarium* isolate no. 90 in relation to temperature.

Discussion

The plasticity of the morphological characteristics of the Gladiolus Fusaria under different cultural conditions makes it difficult or impossible to compare written descriptions when conditions of culture are not adequately specified. Wollenweber & Reinking's (1935) system of classifying the Fusaria, particularly of the section *Elegans*, depended on taxonomic differences which are greatly influenced by environment or which can be found within any one isolate on single sporing and the present work emphasizes the need for strict standardization in work relating to Fusarium taxonomy.

The extremely variable nature of the members of the section *Elegans* of the Fusaria can be explained in three ways:

(*a*) High mutation rate when taken into culture, as postulated by many authors and dealt with specifically by Miller (1946).

(*b*) Origin of new types by formation of heterocaryons between already existing variants or between spontaneous mutants (Buxton, 1954).

(*c*) Changes in relation to cultural environments, as described above.

Variation brought about by method (*a*) would presumably be permanent, whereas those due to (*b*) and (*c*) would fluctuate, depending on degree of selection by environment. Selection may act upon nuclei, as in heterocaryosis, or on genetic structures in the cytoplasm in variants arising from uninucleate haploid spores in culture. The combined effects of mechanism (*a*) and (*b*) would account for some of the origins of new strains differing in their pathogenicity towards different hosts and in their degrees of virulence.

The changes in spore shape, size and mode of presentation, colony colour, and type of aerial mycelium, obtained in response to changes in cultural conditions suggest that it will be difficult to maintain the separation of *Fusarium orthoceras* Woll. var. *gladioli* McCulloch and *F. oxysporum* Fr. var. *gladioli* Massey. Moreover, a wide range of morphological types (which grow under standard conditions) can be obtained from Gladiolus plants showing the same disease syndrome.

Evidence has been obtained (unpublished work) that heterocaryons can be formed between different pathogenic strains of the Gladiolus Fusaria (isolates 87 and 90; cf. Pl. 5, fig. 5, Buxton, 1955). Genetic markers were produced by ultraviolet irradiation of spores, resulting in biochemical deficiency of the irradiants. Heterocaryons were readily formed between an arginine-requiring mutant of isolate 90 and a paraminobenzoic acid-requiring mutant of isolate 87. Investigations of changes in pathogenicity of single biochemically deficient isolates, and of the heterocaryons they form, should help to elucidate the mechanism of the origin and stability of new physiological races within the section *Elegans*. These facts, together with the demonstration (Buxton, 1955) that the same organism can produce symptoms of Fusarium Yellows and Fusarium Corm Rot consecutively within one cultivar and/or separately in distinct cultivars, and the demonstration (Buxton, 1954) that heterocaryons can be formed between isolates which under standard cultural conditions correspond with *F. oxysporum* var. *gladioli* and *F. orthoceras* var. *gladioli*, suggest that all the isolates of *Elegans* Fusaria from diseased Gladioli can probably best be accommodated in *F. oxysporum* Fr. f. sp. *gladioli* (Massey) Snyder & Hansen.

My thanks are due to the Agricultural Research Council for a Research Studentship during the tenure of which this work was carried out, and to Dr N. F. Robertson for supervision.

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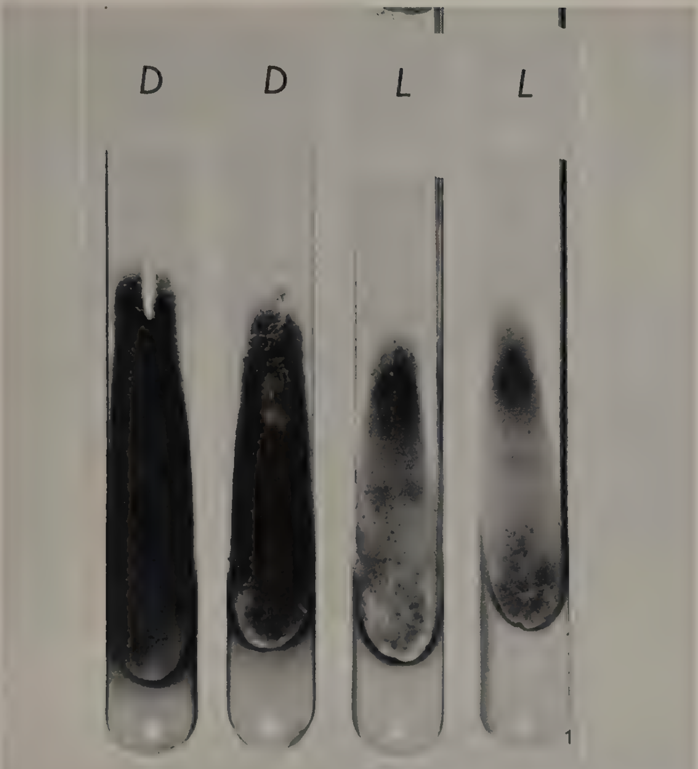
EXPLANATION OF PLATE 6

Fig. 1. Pairs of cultures of isolate 87 (all of common single-spore origin) compared after 20 days' exposure to fluorescent lamp, *L* in light, *D* in dark. Pionnotes and sclerotia are seen in *L*.

Fig. 2. Macroconidia of cultures of common origin retained 1 day in light (*a*), and 20 days in light (*b*).

(*Accepted for publication* 24 *October* 1954)



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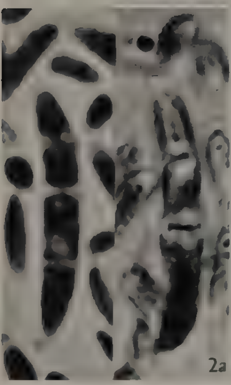

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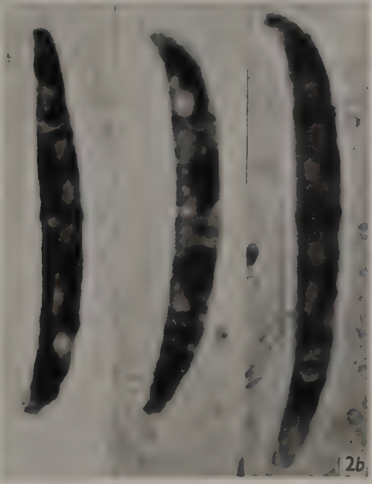

2b

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Trans. Brit. mycol. Soc. **38** (3), 213–216 (1955).

NEPHROMIUM ARCTICUM (L.) FR., A LICHEN NEW TO BRITAIN

By D. N. McVEAN
The Nature Conservancy, Edinburgh

(With Plate 7 and 1 Text-figure)

Nephromium arcticum (L.) Fr. is reported for the first time in Britain from three separate localities in the north of Scotland. Morphology and distribution of this species, and of the closely related *N. antarcticum* (Wulf.) Nyl. are briefly described. A few ecological notes are given for each Scottish locality.

On two occasions in the summer of 1953, and once in 1954, the writer collected a yellow, foliaceous lichen from moss heath at an altitude of 3000 ft. in the Northern Highlands of Scotland. Although without apothecia the thallus was characteristic enough in appearance to suggest the common circum-boreal species *Nephromium arcticum* (L.) Fr. This identification has since been confirmed.

The thallus of this species is generally large and spreading (6–8 cm. being attained in the Scottish specimens), with a smooth, undulating, greenish to yellowish upper surface. The margin is irregularly divided into rounded lobes. The under-surface is black with paler margins and bears short black rhizoids which attach the thallus closely to the substratum. The apothecia, which have so far not been observed in Scotland, are generally large (4–20 mm.), adnate to the surface of the lobes, and the disk flat and reddish brown. Spores are brownish, oblong-ellipsoid and 3-septate.

N. arcticum is circum-boreal in distribution (Text-fig. 1) and has been recorded from Iceland (Galløe, 1942) and Greenland (Dahl, 1950), but not the Faroe Isles or Spitzbergen. There are southern outliers in the United States (New York and New England; Fink, 1935) and in the Tatra Mountains of Central Europe (Zahlbruckner, 1926).

The most closely allied species of the genus is *N. antarcticum* (Wulf.) Nyl. which has a white underside and a flat-lacunose upper surface, and is peculiar to the Antarctic region.

N. arcticum is found mainly on ground mosses, but also on tree bark, and it enters, as a minor constituent, into many northern heath associations of intermediate altitude, such as heathy birch woods and dwarf-shrub associations (Nordhagen, 1927; Samuelsson, 1917).

SCOTTISH LOCALITIES

Wester Ross

At two stations at about 3000 ft. on a species-rich moss heath forming scattered and spreading patches on the mosses (Pl. 7). The soil, a rich clay developed from certain shaly beds of rock, gave a glass-electrode meter reading of pH 7·1.

Species of the Moss Heath

	Station I (2 m.^{2} plot)	Station II (4 m.^{2} plot)
Principal species (cover > 20 %)	*Rhacomitrium lanuginosum*	*Rhacomitrium lanuginosum*
	Salix herbacea	
Subsidiary species (cover < 20 %)	*Carex bigelowii*	*Carex bigelowii*
	Saussurea alpina	*Salix herbacea*
	Polygonum viviparum	*Polygonum viviparum*
	Festuca vivipara	*Festuca vivipara*
	Armeria maritima	*Armeria maritima*
	Vaccinium myrtillus	*Galium hercynicum*
	Luzula spicata	*Luzula spicata*
	Euphrasia frigida	*Euphrasia frigida*
	Silene acaulis	*Silene acaulis*
	Solidago virgaurea var. *cambrica*	*Deschampsia caespitosa*
	Polytrichum urnigerum	*Thymus drucei*
	P. alpinum	*Polytrichum alpinum*
	Rhytidiadelphus loreus	*Rhytidiadelphus loreus*
	Aulacomnium turgidum	*Aulacomnium turgidum*
	Pleurozium schreberi	*Pleurozium schreberi*
	Dicranum fuscescens	*Hylocomium splendens*
	Ptilidium ciliare	*Cladonia uncialis*
	Lophozia alpestris	*C. bellidiflora*
	L. hatcheri	*Sphaerophorus globosus*
	Sphaerophorus globosus	
	Cladonia uncialis	
	C. bellidiflora	
	Cetraria islandica	
	Peltidea aphthosa var. *leucophlebia*	
	Peltigera canina	
	Nephromium arcticum	
Incidental species (only present outside plot)	*Rumex acetosa*	*Alchemilla alpina*
	Deschampsia caespitosa	*Cetraria islandica*
	Ranunculus acris	*C. aculeata*
	Thymus drucei	*Cerania vermicularis*
	Thalictrum alpinum	*Stereocaulon evolutoides*
	Sedum rosea	*Peltidea aphthosa* var. *leucophlebia*
	Juncus trifidus	*Nephromium arcticum*
	Cladonia sylvatica	

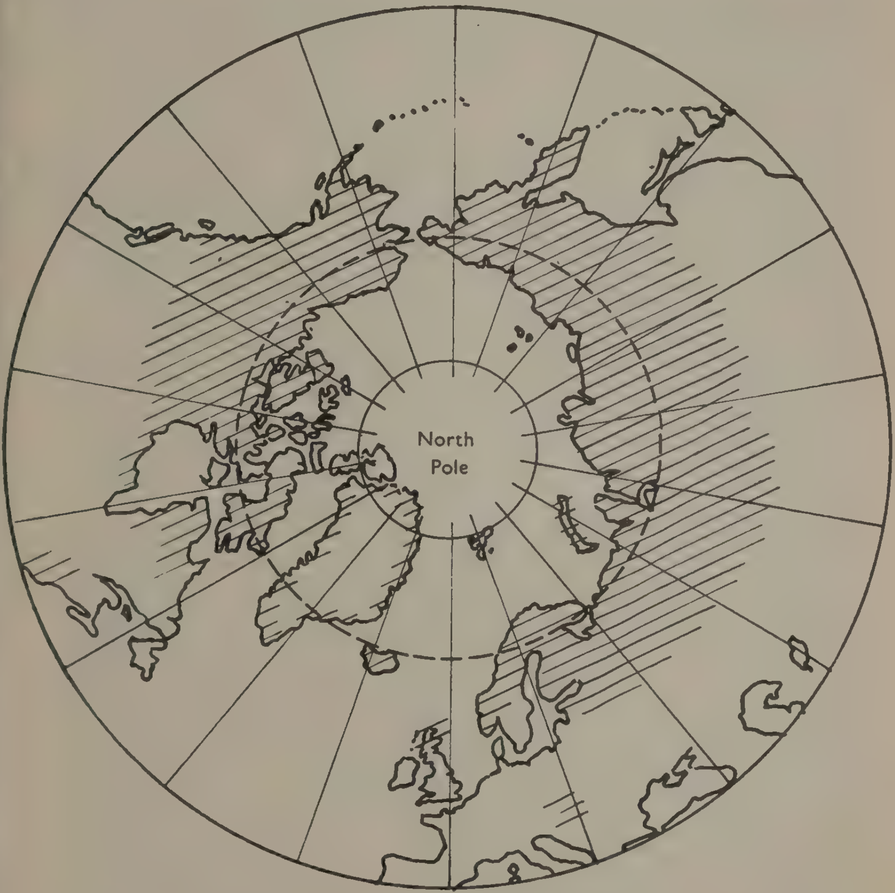
In both localities the moss heath association is broken by solifluxion terraces whose flat tops carry a more open community containing *Sibbaldia procumbens*, *Gnaphalium supinum* and *Cardaminopsis petraea*.

East Inverness-shire

At about 3500 ft. on steep, unstable slopes of coarse mica-schist soil bearing fragments of moss heath, less rich in species than the above, but including *Gnaphalium supinum* and *Sibbaldia procumbens*.

In none of the localities can the *Nephromium* be described as abundant though several scattered patches have been discovered in one of them. Neither is it a species likely to have been overlooked often in the past so that, although possibly present in other areas in the moss heath association, it seems likely to remain one of our rarer ground lichens.

I am indebted to Dr W. Watson and Mr F. A. Sowter for confirming the identification of the lichen and for encouraging the production of this note.



Text-fig. 1. Distribution of *Nephromium arcticum* (L.) Fr.

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EXPLANATION OF PLATE 7

Nephromium arcticum at one of its Scottish localities. The pale expanses of thallus scattered throughout the field are fragments of *N. arcticum*. The darker, branched lichen in the left foreground is *Stereocaulon evolutoides* (Magn.) Frey. Other cryptogams and phanerogams visible are *Rhacomitrium lanuginosum*, *Polytrichum* spp., *Salix herbacea*, *Solidago virgaurea* v. *cambrica*, *Polygonum viviparum*, *Carex bigelowii* and *Festuca vivipara*.

(*Accepted for publication* 1 *November* 1954)

(*Facing p.* 216)

Trans. Brit. mycol. Soc. **38** (3), 217 (1955).

ANCYLISTES PFEIFFERI BECK SENSU BERDAN RENAMED *A. BERDANII* SP.NOV.*

By F. K. SPARROW

University of Michigan, Ann Arbor, Michigan

In Miss Berdan's (1938) revision of *Ancylistes*, *A. pfeifferi* Beck is redefined on the basis of her observations on a parasite of *Closterium areolatum*. She pointed out that Beck (1896) was no doubt dealing with a mixture of *Myzocytium* (probably *M. megastomum*) and *Ancylistes*. This is clearly evident from his description. It is also supported from the fact that Miss Berdan frequently found representatives of the two genera associated, as has this writer, in the same host cell.

Since Article 64 of the International Rules clearly specifies that species based upon mixtures must be rejected, such action is proposed here with respect to Beck's species. *A. pfeifferi* Beck is therefore rejected and Miss Berdan's fungus may appropriately be termed *A. berdanii* sp.nov. The new species is therefore validated by the following Latin diagnosis based on Miss Berdan's description (Berdan, 1938, p. 407).

Ancylistes berdanii sp.nov.† (Berdan, 1938, figs. 9–13, as *A. pfeifferi*)

Synonym: *A. pfeifferi* Beck sensu Berdan

Mycelia pars intramatricalis irregularis, 7·5–14μ crassa (saepissime 9·5–11μ) ante septationem, apicibus liberis incrassata et irregulariter secus hyphas, segmentis vel cellulis forma femori similibus, 43–67μ longis, 10·5–11·5μ crassis ad mediam, 13–19μ ad apices incrassatos, 9·5–12·5μ (plerumque 10·5–12·5) ad septa, vacuolatis. Hypharum exteriorium segmenta 4–7μ × 44·5–104μ, plerumque 45–70μ longa. Conidia hyalina, globosa, 21–23·5μ in aere, 22·2–30μ (papilla includente) sub aqua; tubo germinali 4·5–5 mm. Appressorium disciforme, 3–4μ, ad ultimum applanatum et vagina hyalina gelatinosa praeditum, ea retroversa ad partem crassiorem cellulae infectionalis. Cellula infectionalis 7·5–13μ × 37–67μ, plerumque 11–12μ × 40–55μ. Cellula penultima saepe brevissima lataque, 6–10μ × 9–26μ. Conjugatio vel lateralis vel scalariformis. Gametangia ex cellulis vegetativis a septis 1 vel 2 pro positione terminali vel mediali; mascula recta, angusta, interdum plurifariam ramosa si lateraliter conjugata, ramis obtusis; femina distenta, cellula zygosporam includente verticillatim umbonata, protrusionibus 9–10 crassis brevibus 9–10μ latis, obtuse truncatis basi singulatim in cellulis brevibus stipitiformibus aequinumerosis sedentibus. Zygospora globosa, 18·5–22μ diam., laevis, brunnea, libera in cellula gametangii, membrana crassa. Germinatio hucusque ignota. Species parasitica in *Closterii aerolati*, Chapel Hill, Carolina boreali.

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* Contribution from the Botany Department, University of Michigan, no. 1029.
† I am indebted to Prof. H. H. Bartlett for the Latin diagnosis.

(*Accepted for publication* 1 *October* 1954)

Trans. Brit. mycol. Soc. **38** (3), 218–220 (1955).

AN AUSTRALIAN RECORD OF *CEPHALIOPHORA TROPICA*

By F. M. CROOK* and W. R. HINDSON

Defence Standards Laboratories, Melbourne, Australia

(With Plate 8)

Cephaliophora tropica Thaxter is reported for the first time in Australia. It has been isolated from painted steel panels and from a variety of organic materials, and is now well established in the vicinity of Maribyrnong, Victoria.

Cephaliophora tropica Thaxter (Moniliaceae) was first described by Thaxter (1903). He isolated it from mongoose dung found near Kingston, Jamaica, and in the same paper reported it from other diverse areas in or near the tropics, always from the dung of animals. It does not appear to have been reported again until found by Wolf (1949) in Oxford, North Carolina, on decaying tobacco leaves that had been in contact with soil.

In September 1949, we isolated a fungus new to us from painted stainless steel panels that were being tested for resistance to fungal growth. The test method consisted in placing individual panels in glass boxes on a malt agar medium, and inoculating the medium and panels with a mixed spore suspension containing spores of *Memnoniella echinata*, *Aspergillus flavus*, *A. amstelodami*, *A. niger*, *Penicillium luteum*, *P. notatum* and *Rhizopus stolonifer* (*R. nigricans*). The boxes were incubated for 28 days at 30° C. and 90–100 % relative humidity, and the amount of fungal growth on the painted surfaces assessed visually. Since the tests are not done under sterile conditions, organisms other than those in the inoculum sometimes develop on the medium or test panels.

In one series of tests on a glossy phenolic enamel, a contaminating fungus was observed on several panels, in macroscopic appearance rather like streaks of rust (Pl. 8, fig. 1). On the test panels, the mycelium, mostly aerial, could be seen scrambling over the painted surface without apparently damaging it, as white cottony hyphae, somewhat fasciculated (Pl. 8, fig. 2). The sporing heads were more or less crowded into irregular groups (Pl. 8, fig. 3) and were pinkish brown in colour.

The fungus when inspected at high power under the microscope was readily identified as *Cephaliophora tropica* Thaxter. Microscopic preparations made from a culture of the fungus on malt agar showed it to correspond closely to the descriptions given by Thaxter and Wolf.

Observations of the freshly isolated fungus on malt agar showed that on this medium the mycelium is white and cottony, mainly aerial, and rapidly growing. Conidiophores are erect, broad and short. The fertile vesicle is globose, usually borne on a pedicel often only slightly less in length and breadth, the whole thin-walled and hyaline (Pl. 8, fig. 4), or sometimes the fertile vesicle may be sessile, a globose protuberance arising directly from a hypha (Pl. 8, fig. 5). Spores are borne all over the vesicle, initiated simultaneously as more or less obovoid hyaline buds (Pl. 8, fig. 6). The spores grow outward in all directions, rapidly becoming ellipsoid, coloured, and loculate, the septum nearest the point of attachment usually appearing first (Pl. 8, fig. 4). The spores are attached to the vesicle by a pedicel, a tapering prolongation of the colourless basal locule. At maturity the spores are 3-, 4-, occasionally 5-septate, thick-walled, smooth, bright yellow-brown, 8 to 18 in a head, and crowded, the conidiophore often being almost completely hidden. Measurements of the spores in Amann's lactophenol ranged from 29 to 57 μ by 15 to 20 μ, averaging 40 μ by 17 μ, or slightly larger than reported by Thaxter or Wolf, but roughly within the maximal size given by Thaxter (about 50 μ by 20 μ). Germination takes place in 8–16 hr., from one or more loculae; it appears that all loculae may be fertile, including the basal locule which Thaxter reported as sterile (Pl. 8, fig. 7).

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*Now at Australian Paper Manufacturers Ltd., South Melbourne.

After a year in culture, the fruiting parts were rather more variable, particularly the spores, some of which were produced in a Y-shaped form resembling that described by Thaxter for *C. irregularis*, others having a pronounced curve (Pl. 8, fig. 8). There was, however, much less variability than Thaxter had observed in his 12-year-old culture. Our isolate has now been in culture for 5 years and has remained true to type. Normal conidia are always present but occasionally we have noticed outbursts of the Y-shaped or curved conidia. This could suggest that Thaxter's *C. irregularis* may not perhaps be a separate species.

In one culture on potato-dextrose agar that became contaminated with a bacterium, another abnormality was observed. There were produced heads of giant spores (Pl. 8, fig. 9), much elongated and often many-septate, but otherwise normal, and borne on normal conidiophores. The largest measured about 70 μ, and had reached this length before darkening of the walls or septation was complete.

As we are particularly interested in the effects of fungi on cellulosic materials, attempts were made to grow the fungus on cotton duck strips under our usual testing conditions for determining the resistance of fabrics to fungal attack (Standards Association of Australia, 1944). After 28 days' incubation, twice the normal time, there was still no sign of fungal growth on the test strips, and breaking strength determinations showed that there had been no loss of strength. It is concluded, therefore, that this strain of *C. tropica* has no cellulolytic power.

This record is apparently the first report of *C. tropica* in Australia. The fungus now seems to be well established in the vicinity of this laboratory; it has occurred on several occasions as a contaminant on a variety of organic test materials in tests for fungal resistance. The occurrences reported by Thaxter and Wolf and in this paper suggest that it is a soil form, far from strictly coprophilous, and as Thaxter suggests, quite widespread. It is somewhat surprising that it has not been reported more often, especially as it is, as Wolf points out, 'strikingly different...from all other capitate Moniliales'.

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EXPLANATION OF PLATE 8

Fig 1. *Cephaliophora tropica* growing over the surface of a stainless steel panel painted with a glossy phenolic enamel. × ⅜.

Fig. 2. As for fig. 1, showing cottony nature of hyphae. × 11.

Fig. 3. Sporing heads of *C. tropica* on paint panel. × 30.

Fig. 4. *C. tropica* on malt agar—fertile vesicle borne on pedicel. × 180.

Fig. 5. *C. tropica* on malt agar—fertile vesicle without pedicel, arising directly from hypha. × 180.

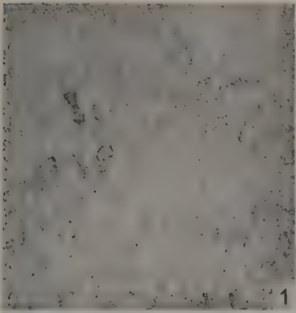
Fig. 6. *C. tropica* on malt agar—initiation of spores on vesicle. × 400.

Fig. 7. Germination of spore of *C. tropica* on malt agar showing fertility of basal locule. × 320.

Fig. 8. Y-shaped and curved spores of *C. tropica* in culture for a year. × 490.

Fig. 9. Head of giant spores produced in a culture of *C. tropica* on potato-dextrose agar contaminated with a bacterium. × 490.

(*Accepted for publication* 10 *November* 1954)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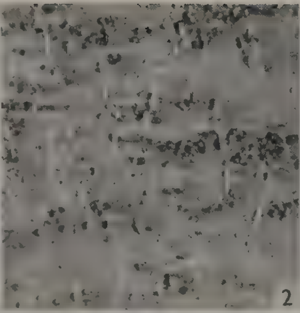
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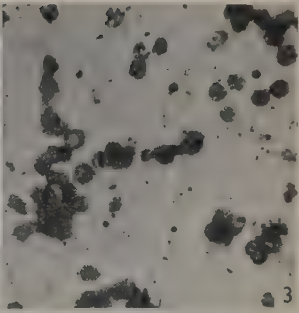
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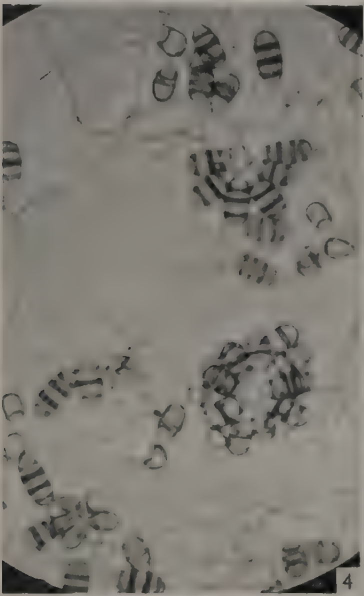
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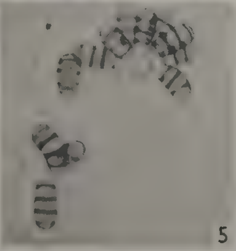
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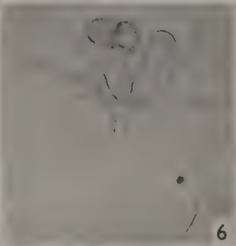
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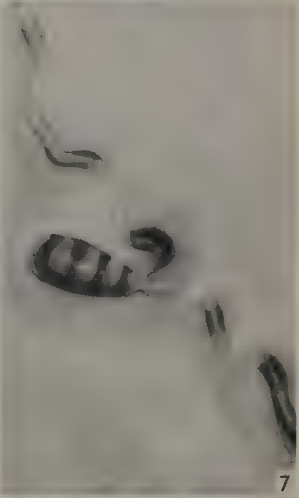
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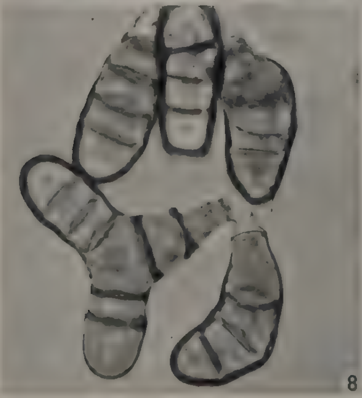
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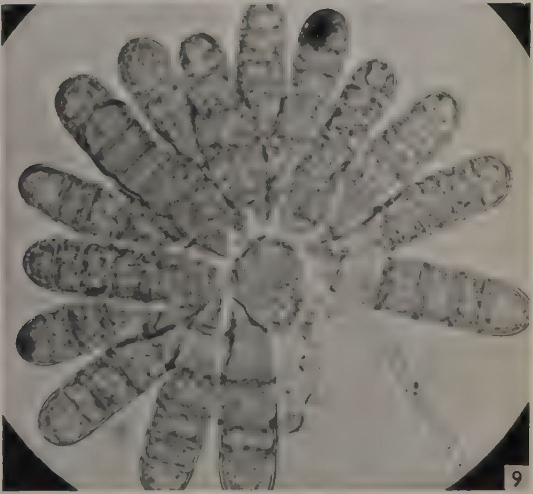
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Trans. Brit. mycol. Soc. **38** (3), 221–239 (1955).

SEASONAL INCIDENCE OF *SPOROBOLOMYCES* ON CEREAL LEAVES

By F. T. LAST

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(With 7 Text-figures)

The numbers of *Sporobolomyces* colonies developing on leaves of three crops (winter- and spring-sown wheat and spring-sown barley) were estimated by sampling at 14-day intervals, using a technique that selects fungi that grow on artificial media and whose spores are forcibly discharged.

After the internodes elongated and the crops became dense, the changes in numbers of *Sporobolomyces* were similar in all three crops; leaves produced few colonies until they had lived half their lives, when the number of colonies progressively increased, to reach a maximum after the leaves died. Before the internodes elongated and the crop was still sparse, winter-sown wheat also produced colonies on living leaves, but colonies occurred only on dead leaves in still sparse spring-sown wheat and barley.

Dead leaves in summer had three times as many colonies per unit area as dead leaves in winter. The upper and lower surfaces of ageing leaves carried equal numbers of colonies, but the distal parts carried more than the proximal. The number of colonies seemed to be correlated with a high humidity that enables *Sporobolomyces* to spread and, perhaps, with exudations from ageing leaves of substances that encourage its growth.

The occurrence of *Sporobolomyces* colonies seemed not to influence the development of leaf parasites or to be influenced by them, but *Tilletiopsis*, another innocuous fungus that occurred only in July and August, greatly decreased the number of colonies of *Sporobolomyces*, particularly on the spring-sown barley. In addition *Cladosporium* was regularly isolated from dead leaves, more abundantly during summer than winter, and *Bullera* sporadically. It is suggested that, as with roots and the 'rhizosphere', leaves have a 'phyllosphere', with a characteristic micro-flora that may contain many species.

Twenty isolates of *Sporobolomyces* and ten of *Tilletiopsis* were identified as *Sporobolomyces roseus* Kluy. et van Niel and *Tilletiopsis minor* Nyland, respectively. The isolates of *Bullera* were found to be *B. alba* (Hanna) Derx.

Introduction

The spores of *Sporobolomyces* are at times the most numerous type in the air (Hirst, 1953). Derx (1930) found that many *Sporobolomyces* colonies developed on Petri dishes containing a layer of gelatine exposed in a field of ripe wheat, and that the spores came from colonies of the fungus on the wheat leaves. He also isolated colonies of *Tilletiopsis*, a fungus since isolated from a range of mono- and dicotyledonous plants (Nyland, 1950).

Sporobolomyces and *Tilletiopsis* both form spores which are forcibly discharged. *Sporobolomyces* ('Pink Yeast') multiplies by budding and by spores produced asymmetrically on sterigmata; the spores are discharged by the drop-excretion method typical of Hymenomycetes (Buller, 1933). *Tilletiopsis*, after first reproducing by budding, develops a branched mycelium bearing sickle-shaped spores (Nyland, 1950). Little is known of the nuclear behaviour of these fungi, but they are usually considered to belong to the Heterobasidiomycetes (Olive, 1953).

Although these fungi are known to occur on leaves, the conditions that favour their development, and whether or not they influence susceptibility to pathogens, have not been studied. The work described below was designed to gain information on these points.

ISOLATION OF *SPOROBOLOMYCES* FROM LEAVES

The method used was similar to those described by Derx (1930) and Buller (1933). It has two phases: (1) the exposure phase during which spores produced by colonies on the leaf fall on to agar and start budding; and (2) the incubation phase during which budding continues until colonies become visible.

Leaf blades are laid across the lower half of Petri dishes containing 10 ml. of potato-dextrose agar (P.D.A.) and are supported on the rims when the lid is replaced. Before the leaf is removed at the end of the exposure phase, its area exposed above the agar is calculated by multiplying its length by the mean breadth measured at the positions where the leaf rests on the rim of the lower half dish. The dishes are then incubated until the colonies can be counted. The dishes are inverted during the incubation phase to prevent colonies developing on the agar from spores falling from colonies formed on the lid during the exposure phase by contact with the leaf surface not being examined.

Experiments were made to find the optimum temperature and duration of both the exposure and incubation phases.

Exposure phase. Leaves were kept over P.D.A. for 24 hr. at different temperatures, then all the Petri dishes were incubated at 22·5° C. and the colonies counted 2–3 days later. Fig. 1 B records two experiments in which results are expressed as the numbers of colonies per cm.^{2} of leaf exposed. The data for Exp. I are the means of twenty-two experiments done on different occasions during June–August with leaves taken from different positions on the main stems of three wheat crops. A similar variety of leaves was used in Exp. II, but the tests were made in August only. With increasing temperature above 6·5° C., the number of colonies increased, reached a maximum at about 20° C. and then decreased almost to zero at 30° C. There was no statistically significant difference between the number of colonies at 15, 20 and 22·5° C.

As 22·5° C. is the minimum which can be easily maintained throughout the year in ordinary incubators, it was the temperature adopted for the exposure phase. The optimum duration of the exposure phase at 22·5° C. was determined by exposing leaves for 6, 12 and 24 hr., and then varying the period of incubation so that the total time at 22·5° C. was 72 hr. Fig. 1 A compares the results of such a test with one done at 10° C. At 22·5° C. the colony number increased rapidly during the first 12 hr., but increased little afterwards, whereas at 10° C. it increased greatly during the next 12 hr.

For convenience of routine sampling the leaves were exposed above agar overnight, from about 16.00 G.M.T. to about 09.30 G.M.T.—approximately 17·5 hr.

Incubation phase. Slopes of P.D.A. were point-inoculated with a *Sporobolomyces roseus* isolate from wheat, and incubated at four temperatures. After 13 days, the colonies were suspended in water and the cells counted in a haemocytometer. There were 2·2 × 10⁹, 5·3 × 10¹⁰, 7·7 × 10¹⁰ and 0 cells per slope at 3, 10, 22·5 and 30° C., respectively. As 22·5° C. favoured growth it was also adopted for the incubation phase.

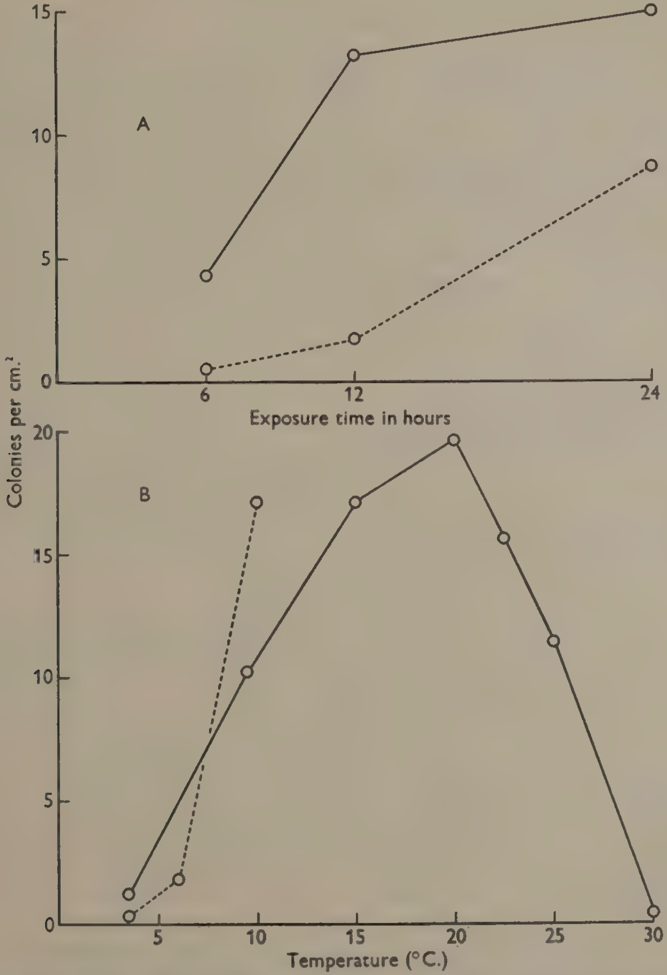

Fig. 1. (A) The number of *Sporobolomyces* colonies developed when leaves were held above potato-dextrose agar (P.D.A.) for varying periods. Temperature during exposure phase: O—O = 22·5° C., and O- - -O = 10° C. (B) The number of *Sporobolomyces* colonies developed when leaves were held above P.D.A. at different temperatures. O—O, Exp. I; O- - -O, Exp. II.

A total of 66 hr. exposure and incubation at 22·5° C. was needed before the colonies developed to a size suitable for counting. The isolation technique adopted, therefore, was: (1) exposure phase 18 hr. (overnight) at 22·5° C.; and (2) incubation phase 48 hr. at 22·5° C.

SAMPLING METHODS

Leaves on reference plants in each crop sampled were labelled, to supply standards from which the age of any leaf on unlabelled stems could be found. At each sampling, eight main stems were taken and their leaf blades stripped off. The leaves were numbered in order of emergence; thus the first leaf to emerge was leaf 1, the second was leaf 2, and so on. The upper surface of half the leaves < 12 cm. in length from each position and the lower surface of the other half were exposed above P.D.A. The leaves from any one stem were alternated so that if the upper surface of leaf 1 was exposed, the lower surface of leaf 2 was exposed.

Leaves > 12 cm. in length provided four exposure positions—all the combinations of the upper and lower surfaces with the proximal and distal areas of the leaf.

The number of *Sporobolomyces* sp. colonies per cm.^{2} produced by replicates varied considerably and for statistical analysis the numbers were transformed by a method described by Kleczkowski (1949). As many leaves gave no colonies the transformation used was $\log(n+10)$, where n = actual number of colonies per cm.^{2} The data given in this paper, unless otherwise stated, are the detransformed means.

RESULTS OF SYSTEMATIC SAMPLING OF FIELD CROPS

Three crops, winter-sown wheat, spring-sown wheat and spring-sown barley, grown at Rothamsted Experimental Station (R.E.S.) were sampled at approximately 14-day intervals from emergence to the death of the flag leaf—the last leaf to emerge before the ear. There were two strips of the winter-sown wheat, one given fertilizer and the other not.

The weekly mean rainfall and maximum and minimum air temperatures during the lives of the three crops, and the life-span of each leaf on the main stem of each crop are plotted in Fig. 2. The life-span of a leaf extended from the first occasion it was visible to the first occasion it was observed dead.

WINTER-SOWN WHEAT (BROADBALK FIELD, R.E.S.)

Wheat has been grown continuously since 1843 on this field, which is divided into 18 strips receiving different fertilizers. Plots 3 and 8 only were sampled. Plot 3 has received no fertilizers since 1839; plot 8 is given yearly 3·5 cwt./acre superphosphate, 2 cwt./acre sulphate of potash, 1 cwt./acre sulphate of soda and 1 cwt./acre sulphate of magnesia at drilling, and 6 cwt./acre sulphate of ammonia as a spring top dressing. Each plot is divided into 5 sections, one of which is fallowed every year. In each year, therefore, all plots contain crops growing 1, 2, 3 and 4 years after fallow. Plants were sampled only from the first crop after fallow.

Wheat, variety Squareheads Master 13/4, was sown on 23 October 1953 and had emerged by 9 November on both plots. Tillering started in plot 8 at the beginning of December, but the fertilizers did not obviously affect the density of the crop until April. Crop density was not measured, but Watson (1947) gives the following data from his experiment

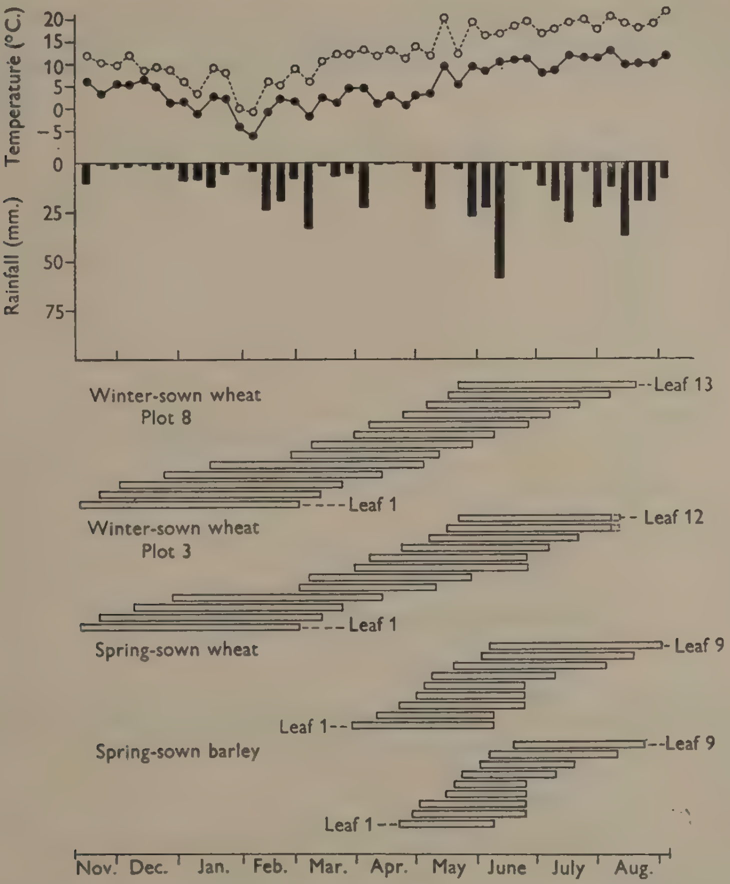

Fig. 2. Weekly mean rainfall and maximum and minimum air temperatures at Rothamsted Experimental Station from November 1953 to August 1954, and the life-spans of the main stem leaves of the crops sampled. ○- - -○, weekly mean maximum air temperature; ●—●, weekly mean minimum air temperature.

done on plants from plots 3 and 7 during the season 1938–9: plot 7 receives 4 cwt./acre of sulphate of ammonia instead of 6 cwt., but otherwise is fertilized as plot 8. The shoot number per metre of row was 67 and 120, and the leaf area per shoot 29 and 45 cm.^{2} for plots 3 and 7, respectively. The data are the means of observations made between late April and the time of ear emergence in June. The differences between plots 3 and 8 in 1953–4 were probably at least of the same order. In this season, the internodes had started to elongate by the end of April on plot 8 which was slightly ahead of plot 3, and 12 and 13 leaves were produced on the main stems of plants in plots 3 and 8, respectively.

Three fungal leaf parasites, *Erysiphe graminis*, *Puccinia glumarum* and *P. triticina*, occurred on both plots, but the intensity of attack was never more than slight.

Fig. 3 shows the successive changes in the number of *Sporobolomyces* colonies per cm.^{2} of leaf area plotted against the stage in the leaf's life, expressed as a percentage on a scale in which the 0 and 100 % points coincide with the first occasions on which the leaf was observed emerged and dead respectively. The data are the means of all the leaves on the main stem.

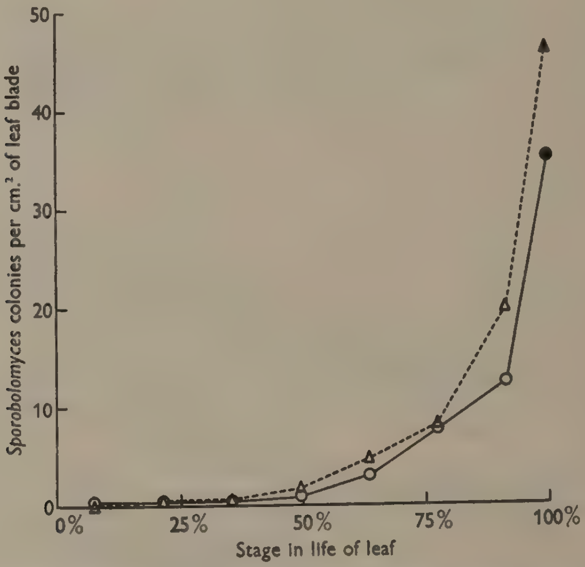

Fig. 3. The successive changes in the number of *Sporobolomyces* colonies per cm.^{2} of leaf blade plotted against the stages in the leaf's life. The data are the means of the main stem leaves taken from winter-sown wheat grown on Broadbalk Field, R.E.S. ○—○, plot 3; △- - -△, plot 8; ○ and △, leaves alive; ● and ▲, leaves dead.

The graphs for the two plots are similar, the values being generally higher in plot 8. During the first half of each leaf's life there were few *Sporobolomyces* colonies, but then the number rapidly increased. This increase was possibly associated with the senescence of the leaf for, although visible changes such as yellow flecking were not detected before the 75 % stage, internal changes had probably already occurred.

The leaves produced on the main stem can be arranged in two groups by their length of life. Thus leaves 1 to 4 inclusive of plants in plot 3 and leaves 1 to 5 in plot 8 lived approximately 110 days, the remainder of the leaves lived about 80 days. In Fig. 4 the successive changes in the number of *Sporobolomyces* colonies per cm.^{2} are plotted against the actual age of leaf

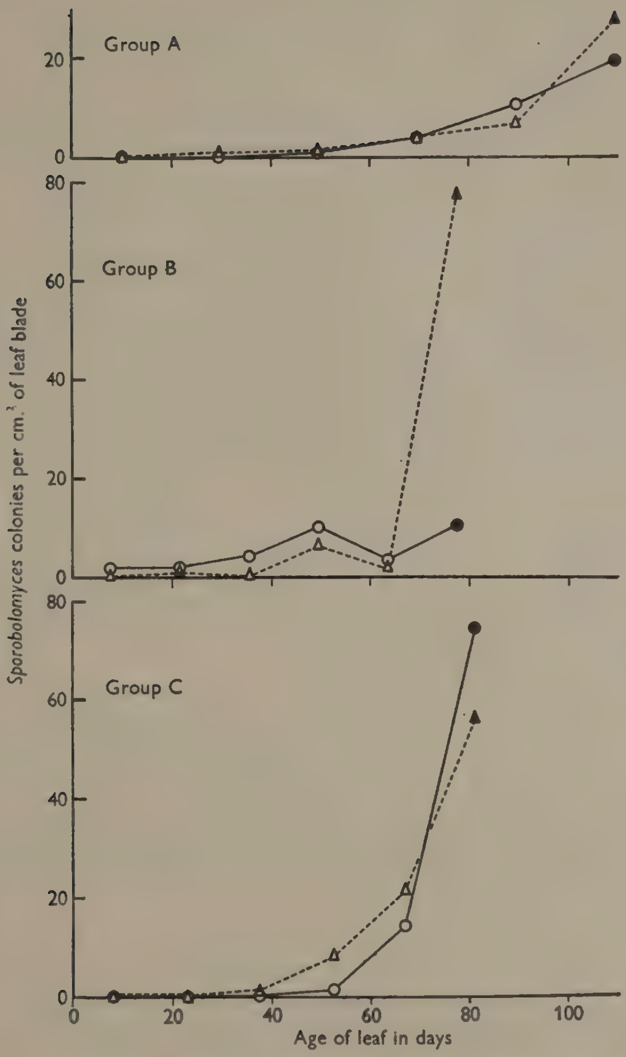

Fig. 4. The successive changes with age in the number of *Sporobolomyces* colonies per cm.² of leaf blade of three groups of main stem leaves taken from plants grown on plots 3 and 8, Broadbalk Field, R.E.S.

Group	Plot 3	Plot 8
A	Leaves 1 to 4	Leaves 1 to 5
B	5 and 6	6 and 7
C	7 to 12	8 to 13

○—○, plot 3; △---△, plot 8; ○ and △, leaves alive; ● and ▲, leaves dead.

in days for three groups of leaves. Group A contains leaves of 110 days' duration. Leaves of 80 days' duration have been subdivided into groups, viz. group B with leaves 5 and 6 (plot 3) and 6 and 7 (plot 8), and group C with leaves 7 to 12 (plot 3) and 8 to 13 (plot 8).

The curves for the leaves of groups A and C, but not B, show the same regular increase in colony number as plotted for the means of all leaves in Fig. 3. In group A, plants from plots 3 and 8 do not differ; colonies were very few until the leaves were 60 days old, and afterwards reached maxima of only 19 and 27 per cm.^{2}. In group C, however, the colony number increased greatly after 45 days in plot 8 and 50 days in plot 3, and the maxima were 56 and 74 per cm.^{2}. There is a longer interval between leaf emergence and increase in colony number in group A than in group C, but as leaves in group A live longer, the change occurs at about the same stage of development in both. The higher maxima with group C than with group A probably reflect the greater amount of inoculum during the spring and summer than in the winter. The amount of inoculum may be affected by three factors, all of which favour a higher concentration during the spring and summer than in the winter. First, even if the leaves were equally heavily contaminated throughout the year the number of colonies per unit length of row would be greater in the spring and summer when there is a greater leaf area per unit length of row than during the winter. Secondly, the higher temperatures from April onwards (group C) favour spread of *Sporobolomyces* (see *Exposure phase*, p. 222). Finally, air movements are less likely to remove spores from a dense crop (group C) than from a sparse crop (group A).

In group C, plot 8 has consistently more colonies per cm.^{2} than plot 3 during the period 40–70 days after leaf emergence. There are more colonies on the dead leaves of plot 3 than of plot 8, but this difference is not significant. The number of colonies can be estimated accurately when there are less than 10 per cm.^{2}, but counting errors increase as the number of colonies increases. In Table 1, the full data obtained at one sample occasion is given. There were only a few days' difference between the ages of the comparable leaves, viz. 8 (plot 3) and 9 (plot 8)...12 (plot 3) and 13 (plot 8).

Table 1. *The distribution of* Sporobolomyces *colonies on wheat leaves taken from plots* 3 *and* 8, *Broadbalk Field, on* 25 *June* 1954

	Leaf number					
	8	9	10	11	12	13
	Geometric means of the actual number (*n*) of colonies per cm.^{2} of leaf blade					
Plot 3	64·1 (D)	15·7	2·3	0·7	0·0	—
Plot 8	—	47·5 (D)	41·3	20·2	10·0	0·7
	Mean log of transformed data ($n+10$)					
Plot 3	1·87	1·41	1·09	1·03	1·00	—
Plot 8	—	1·76	1·71	1·48	1·30	1·03

D = leaf dead.

Difference between means for $P = 0·05$, $= 0·18$.

The statistical analysis of the transformed data shows that, except for the youngest and oldest, the leaves from plot 8 had significantly more colonies than leaves from plot 3. The change on the main stem from the lightly contaminated upper leaves to the heavily contaminated lower leaves shows that the factor determining severity of contamination on any one plant is the age of the leaf.

The differences between comparable leaves in group C from plots 3 and 8 are not an effect of age but probably of crop density, already discussed when comparing groups A and C. Watson found the mean leaf area per metre of row from April to June 1938 to be 17 and 46 dm.^{2} for plots 3 and 7, respectively. Assuming similar differences between plots 3 and 8 in 1954, plot 8 is likely to have more colonies per unit of row length than plot 3, and the resulting spores are likely to be lost from the crop less rapidly in the dense crop on plot 8 than in the sparse crop on plot 3. The spore content of the air within these crops was sampled at three heights on a humid day, 6 July 1953, using a portable suction trap (Gregory, 1954). Table 2 gives the means of three independent estimates, during each of which air was drawn through the impactor unit at 10 l./min. for 5 min.

Table 2. Sporobolomyces *spores per cubic metre of air within two differently fertilized wheat crops on Broadbalk Field, R.E.S.*

(Sampled 6 July 1953 (humid day) between 11.00 and 12.40 G.M.T.)

	Sampling height from ground (cm.)		
	11	46	80
Plot 3. No manures Crop height, 110 cm.	22,100	7,400	7,300
Plot 8. P, K and high N Crop height, 120 cm.	237,000	170,000	41,000

At each sampling height there were more spores per cubic metre of air in plot 8 than in plot 3, and the greatest concentrations were near ground-level.

The curves of the successive changes in colony number on leaves of group B deviate considerably from those of group C which have a similar life-span (Fig. 4). The colony number did not increase regularly but fluctuated, and was much smaller after 60 days than expected by comparison with group C. On dry days, Gregory (1952) and Hirst (1953) found that the concentration of *Sporobolomyces* spores in the atmosphere reached a very sharp peak about 04.00 G.M.T. High spore concentrations were associated with heavy dews, suggesting that free moisture or high humidities are essential for spore liberation. After the leaves of group B had passed the 50 % stage in their lives, there was a dry period in which only 0·25 mm. of rain fell in 3 weeks (see Fig. 2, 7–30 April). Humidity was also probably kept low by the reduced dew formation caused by a protracted spell of dry weather (Manley, 1952). As high humidity seems necessary for spore dispersal, the difference between the actual (group B) and the expected (group C) curves may reflect a lack of airborne inoculum. There was no period of drought during the lives of leaves in group C. At the end of the dry spell, 3·9 mm. rain fell on 1 May, the number of colonies rapidly increased to 77 per cm.^{2} on the leaves of group B on plot 8, but did not increase in plot 3.

Sporobolomyces is not confined to leaves but was also isolated from ears from both plots on 18 August 1954.

Tilletiopsis was isolated first from the flag and penultimate leaves sampled on 6 August 1954 but the colonies could not be counted. Consequently, the amount of growth made on P.D.A. was measured by eye and was used to indicate the extent of leaf contamination. The colonies did not cover more than 1 % of a leaf's area. More leaves were contaminated in plot 8 than in plot 3—of the eight replicates, 5 and 7 in plot 8 and 1 and 2 in plot 3 of the flag and penultimate leaves respectively were contaminated. At this sampling all except the flag leaves in plot 8 were dead. The flag leaf was sampled again on 18 August, when dead, and only one of the eight replicates was found to be contaminated.

SPRING-SOWN WHEAT (GARDEN PLOTS, GREAT FIELD, R.E.S.)

Wheat, variety Atle, was sown on 6 March 1954 and emerged by 27 March. Tillering started in mid-April and internodal elongation in mid-May. The crop was grown on fertile land and was given no fertilizer.

The successive changes in the number of *Sporobolomyces* colonies per cm.^{2} for each of the nine leaves on the main stem are plotted in Fig. 5. The leaves were only slightly infected by parasitic fungi.

The leaves were divided into two groups—leaves 1 to 5 and 6 to 9. Appreciable numbers of colonies were detected only on dead tissue of leaves 1 to 5, and except for leaf 5, the maximum did not exceed 18 per cm.^{2} No observations were made on the dead leaves 3 and 4 as they could not be definitely identified. The changes in colony number on leaves 6 to 9, however, resemble those plotted in the mean curves for winter-sown wheat (Fig. 3)—the number increased steadily after the 50 % stage in the leaf's life, and the maxima were not less than 37 colonies per cm.^{2}

The data from the winter-sown wheat do not indicate that different leaves differ in their suitability for colonization by *Sporobolomyces*. Therefore the curves of successive changes in colony number of leaves 1 to 5 probably differ from those of 6 to 9 because of differences in the way weather affected the dissemination of *Sporobolomyces*.

The development of a cereal crop can conveniently be split into two stages: (1) the period from emergence to the start of internodal elongation when it is sparse; and (2) from the start of internodal elongation to harvest when it is dense. The daily mean relative humidity of the air at Kew, Surrey (38 km. approx. from Rothamsted), for the months of January, April, July and October over a period of 30 years is 84·5, 74·7, 73·0 and 84·6, respectively (Bilham, 1938). The atmospheric humidity during the early, sparse stage (November–March) of a winter-sown crop is high compared with the humidity later (April–August) or with the humidity in a young spring-sown cereal (April and May). Despite fewer colonies in the winter, the relation between leaf age and the changes in colony number was similar for leaves exposed at all stages in the winter-sown cereal, indicating that conditions were favourable for spore dispersal within the crop throughout its life. It seems therefore that the increased crop density from April onwards counteracted the lower atmospheric humidities.

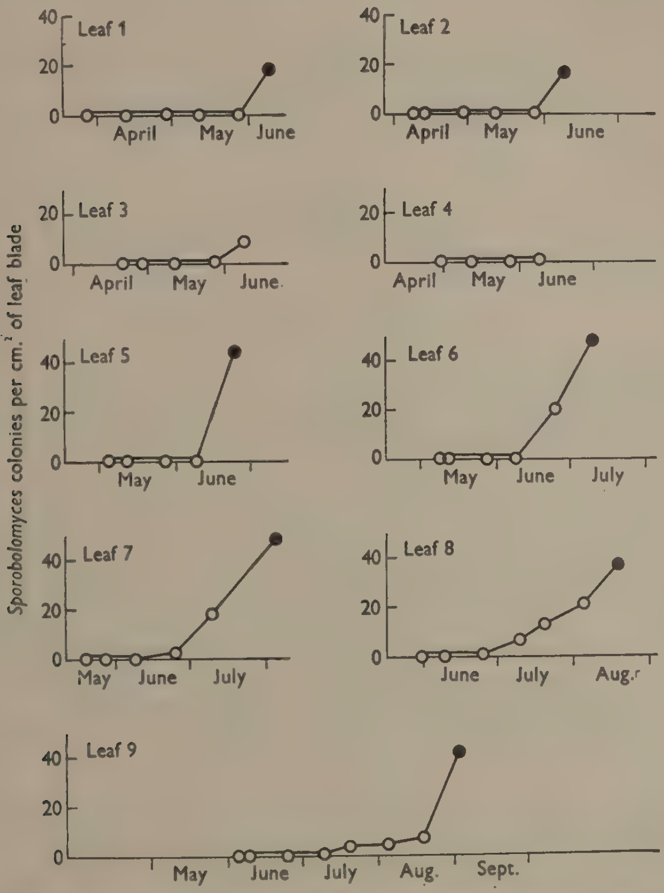

Fig. 5. The successive changes in the number of *Sporobolomyces* colonies per cm.² of leaf blade with age, on each of the nine main stem leaves of a spring-sown wheat crop. ○, leaf alive; ●, leaf dead.

When the spring-sown crop became dense (leaves 6 to 9), the relation between leaf age and the change in colony number resembled that in the winter-sown cereal, but while it was sparse (leaves 1 to 5), the relation was different. As the atmospheric humidity during the life of a spring-sown crop fluctuates about 74 %, the spread of *Sporobolomyces* was probably restricted until the crop became dense enough to raise the humidity.

The curve of successive changes in colony number on leaf 5 resembles the curves for leaves 1 to 4, but the number of colonies isolated from dead tissue, 44 per cm.², is similar to the maxima for leaves 6 to 9. Leaf 5 probably indicates the transition from a sparse to a dense crop.

Tilletiopsis was first isolated from samples taken on 19 July 1954. The maximum area contaminated on any one leaf was 10 %. Table 3 shows the number of leaves, of a possible 8, from which *Tilletiopsis* was isolated on different occasions.

Table 3. *The number of leaves of spring-sown wheat, out of a possible* 8, *contaminated with* Tilletiopsis *on four sample occasions*

	19 July	4 August	18 August	2 September
Leaf 6	0/8 Leaf dead	—	—	—
Leaf 7	6/8	0/8 Leaf dead	—	—
Leaf 8	6/8	8/8	5/8 Leaf dead	—
Leaf 9	2/8	8/8	6/8	3/8 Leaf dead

Leaves alive unless otherwise stated.

Tilletiopsis was isolated most often from leaves 7, 8 and 9, before they died. Thus it was isolated from eight leaves in position 9 on 4 August when the leaves were alive, but only from three on 2 September, soon after the leaves had died.

Spring-sown barley (garden plots, Great Field, R.E.S.)

Barley, variety Plumage Archer, was sown on 5 April in plots within 15 m. of the spring wheat already described. The crop had emerged by 20 April, started to tiller in early May, and the internodes to elongate by the end of May. The plots were given 0·75 cwt./acre superphosphate at sowing and 2 cwt./acre sulphate of ammonia on 27 May. From the end of May onwards the crop was heavily infected by *Erysiphe graminis*.

The successive changes in the number of *Sporobolomyces* colonies per cm.² for each of the nine leaves produced on the main stem are plotted in Fig. 6.

The data in Fig. 6 are similar to those for the spring wheat. Leaves 1 to 5 produced while the crop was sparse had no *Sporobolomyces* colonies on green tissue, whereas leaves 6 to 9 produced when the crop was dense had colonies. Internodal elongation, which starts the change from a sparse to a dense crop, started mid-way through the life of leaf 5. No observations were made on the dead tissue of leaves 2, 3 and 4 as they could not be certainly identified.

The curves for leaves 6 and 9 are similar to the mean curves for the winter crop of wheat, but those for leaves 7 and 8 differ. Instead of the colony number progressively increasing to a maximum on the dead tissue, the maximum occurred before the leaves died. While *Sporobolomyces* colonies declined in number the *Tilletiopsis* colonies increased.

Tilletiopsis was first isolated from the sample taken on 19 July. Table 4 shows the number of contaminated leaves, the severity of contamination and the condition of the leaf on four sampling occasions.

While leaf 9 was alive, the number of contaminated leaves and the area contaminated with *Tilletiopsis* increased with age, but none of the dead leaves was contaminated. This resembles the condition in the two wheat crops, in which the number of contaminated leaves reached a maximum before the leaf died. Leaf 8 was exceptional, for contamination was greatest on the dead leaves. The decline in *Tilletiopsis* may be critically associated with the death of the leaf, so that a difference of a few days between the death of the leaf and taking the next sample may have a large effect. Thus the number of replicates of leaf 7 contaminated and the area contaminated decreased significantly within 8 days (19–27 July). This decrease in *Tilletiopsis* was accompanied by an increase in the number of

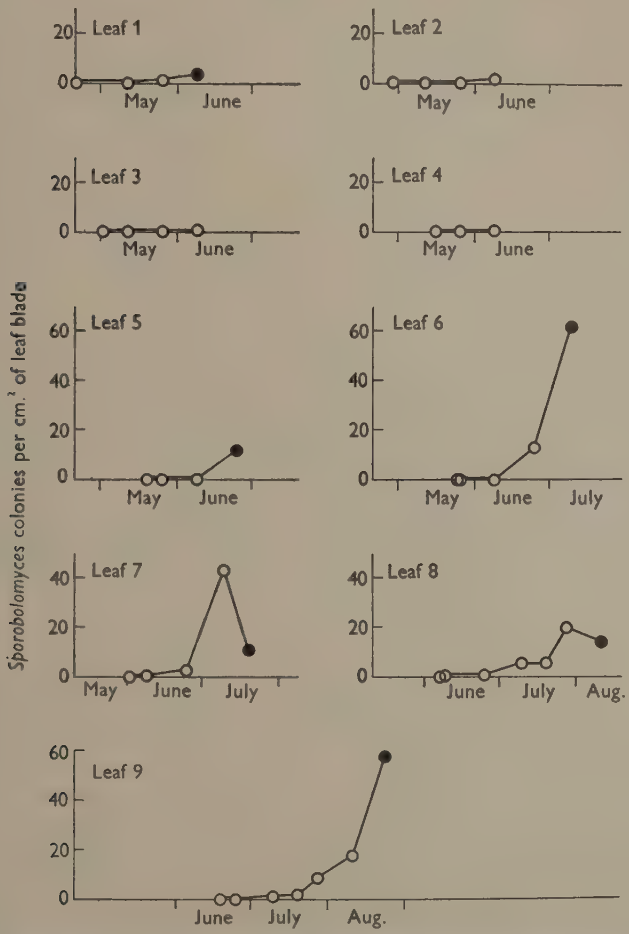

Fig. 6. The successive changes in the number of *Sporobolomyces* colonies per cm.² of leaf blade with age on each of the nine main stem leaves of a spring-sown barley crop. ○, leaf alive; ●, leaf dead.

Sporobolomyces colonies from 10·9 per cm.^{2} on 19 July to 60·0 on 27 July. The second figure is not plotted in Fig. 6, which only shows the number of colonies first recorded on dead tissue.

Table 4. *The distribution of* Tilletiopsis *colonies on barley leaves sampled on four occasions*

	19 July	27 July	10 August	23 August
Leaf 7	7/8+++ Leaf dead	4/8+ Leaf dead	—	—
Leaf 8	8/8+++	8/8+++	8/8++++ Leaf dead	—
Leaf 9	3/8+	4/8+	8/8+++	0/8 Leaf dead
Awns.	—	0/2	5/8+++	—

Denominator = number of replicates in sample; Numerator = number of replicates contaminated.

Severity of contamination: +, up to 1% of leaf area; ++, from 1 to 10% of leaf area; +++, from 10 to 50% of leaf area; ++++, > 50% of leaf area.

Leaves alive unless otherwise stated.

The curves showing the successive changes in the number of *Sporobolomyces* colonies only deviated from the mean curve for the winter-sown wheat when *Tilletiopsis* was isolated abundantly from dead tissue, as with leaves 7 and 8. The changes in *Sporobolomyces* number for leaf 6, which was not contaminated with *Tilletiopsis*, and leaf 9, which was free of *Tilletiopsis* when dead but contaminated earlier, were similar.

Although the plots of spring-sown wheat and barley were within 15 m., *Tilletiopsis* was much more abundant on the barley than on the wheat.

In addition to being isolated from leaves, colonies of *Tilletiopsis* and *Sporobolomyces* were obtained from barley awns.

DISTRIBUTION OF COLONIES ON THE LEAF SURFACE

The traces developing, after exposing leaves heavily contaminated with *Sporobolomyces* above P.D.A., showed a characteristic pattern in which pink colonies tended to be arranged in rows parallel to the veins (Fig. 7). The most definite rows of colonies were usually along the leaf's margins. Table 5 shows the distribution of *Sporobolomyces* colonies between the upper and lower leaf surfaces and between their distal and proximal areas after leaves had passed the 50 % stage in their lives. The data are taken from 59 replicates tested at different times of the year and sampled from three crops, including wheat and barley.

The number of colonies per cm.^{2} was the same on the upper and the lower surfaces, but there were significantly more ($P > 0·05$) on the distal than on the proximal region. The few colonies developed from young leaves were scattered at random.

Contaminated leaves were decolorized in absolute alcohol, stained with cotton blue in lactophenol and examined microscopically. The colonies were superficial; they occurred mainly in the furrows between the veins and on the tissue between the peripheral veins and the leaf's margins. They were not aggregated about the stomata.

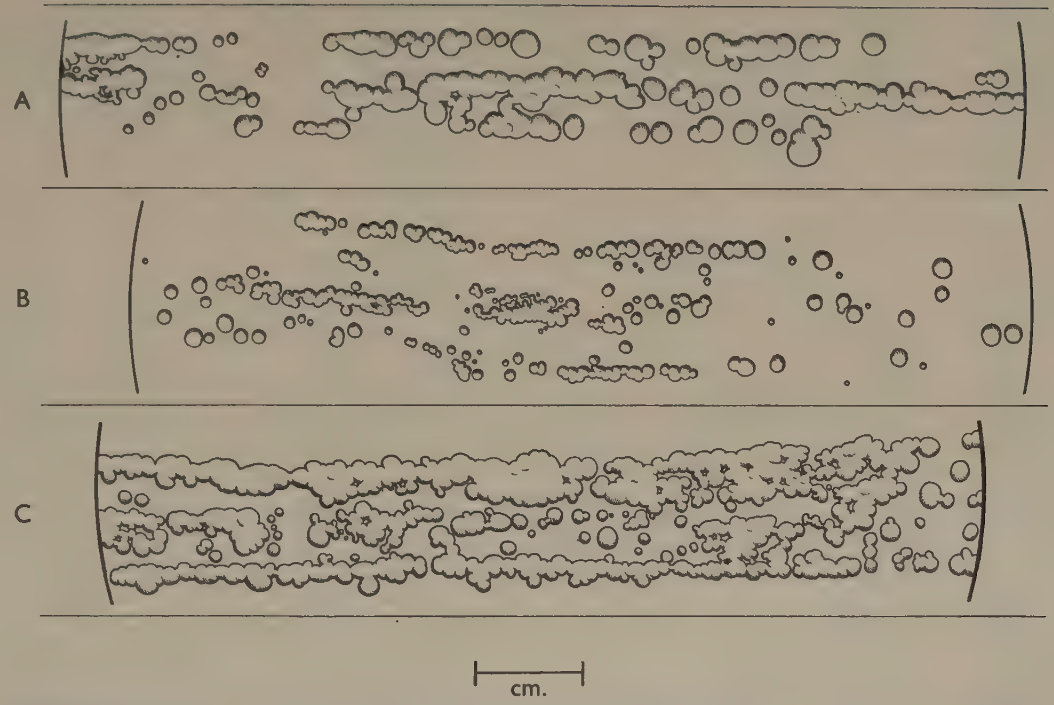

Fig. 7. The arrangement of colonies developed on potato-dextrose agar after exposing 3 wheat leaves A, B and C:

Leaf	Date sampled	Exposure phase	Incubation phase
A	22 July 1954	20 hr. at 22·5° C.	72 hr. at 22·5° C.
B	27 July 1954	24 hr. at 25° C.	48 hr. at 22·5° C.
C	27 July 1954	6 hr. at 30° C. then 18 hr. at 22·5° C.	48 hr. at 22·5° C.

When *Tilletiopsis* was present (July and August), especially on barley, the normal parallel arrangement of *Sporobolomyces* colonies did not develop on agar. During the early stages in a leaf's life, random colonies of *Sporobolomyces* and *Tilletiopsis* were detected, but subsequently mainly *Tilletiopsis* developed over the leaf surface, except for the margins along which many *Sporobolomyces* colonies still developed. The mass of *Tilletiopsis*, which was white, granular, and not arranged in rows, usually enclosed a few *Sporobolomyces* colonies.

Table 5. *The distribution of* Sporobolomyces *colonies between the upper and lower surfaces, and between the distal and proximal regions of wheat and barley leaves*

Totals of transformed data of number of colonies per cm.^{2}

		Distal	Proximal	Totals
Leaf surface	Upper	75·99	73·06	149·05
	Lower	78·93	71·97	150·90
	Totals	154·92	145·03	

Statistical analysis

	Degrees of freedom	Sum of squares	Variance	*F* calculated	*F* from tables
Total	235	19·004			
Distal v, proximal	1	0·409	0·409	5·1	3·9 ($P=0·5$)
Upper v, lower	1	0·009	0·009		
Interaction	1	0·075	0·075		
Error	232	18·511	0·080		

In addition to *Sporobolomyces*, *Cladosporium* was the only fungus regularly isolated on agar. It was isolated from dead tissue and in larger numbers during the summer than winter. When a leaf trace containing *Sporobolomyces* and *Cladosporium* was incubated for more than 66 hr. at 22·5° C., the *Sporobolomyces* colonies were rapidly overgrown.

Colonies of *Bullera* were sometimes isolated. These colonies were similar to those of *Sporobolomyces* but white.

IDENTIFICATION OF ISOLATES FROM LEAVES

As all the *Sporobolomyces* colonies on each sampling occasion appeared to be alike, only twenty isolates, taken on different sampling occasions, were identified. Similarly, as all the *Tilletiopsis* colonies seemed the same, only ten isolates of this fungus were identified.

Derx (1930) describes seven species of *Sporobolomyces* but only one, *S. roseus* Kluyver & van Niel, was identified in the present investigation. *S. roseus*, whose maximum temperature for development is less than 30° C., gives a pink to red mucilaginous colony on potato-dextrose agar at 22·5° C. and produces reniform spores.

Tubaki (1952) separates four species and one variety of *Tilletiopsis* by the colony colour and texture on malt-extract agar. In this investigation only *T. minor* Nyland was isolated—it gives a cream colony which becomes brown and cartilaginous with increasing age when incubated at 22·5° C. on malt agar. The measurements of the curved spores agree with those given by Nyland (1950) for *T. minor*, namely 5·8–14 × 1·5–2 μ, average 9 × 1·8 μ.

Although only thirty isolates were identified, *Sporobolomyces roseus* and *Tilletiopsis minor* probably represent the majority of the *Sporobolomyces* and *Tilletiopsis* colonies, respectively.

The isolates of *Bullera* were identified as *B. alba* (Hanna) Derx. Derx (1930) describes two species differing in the size of the budded cells—the measurements for *B. alba* are 4–5 μ × 2–2·5 μ.

Discussion

Although temperature undoubtedly affects the spread of *Sporobolomyces*, the two main factors governing its incidence on cereal leaves appear to be the age of the leaf and the atmospheric humidity.

Derx (1930) considered that *Sporobolomyces* use sugars exuded on to the leaf surface. That materials favourable for the germination of fungus spores and their subsequent growth are present on plant surfaces was demonstrated by Brown (1922). He found that the electrical conductivity of water drops on petals from an unopened flower bud of *Cerus spectabilis* increased with increasing time of standing, and that this increase in conductivity was positively correlated with an increase in fungal stimulation. In Brown's experiments the materials, some of which were ionic, may have been dissolved from the surface layers. Although much extraneous matter collects on exposed leaf surfaces, Mes (1954) showed that some at least is exuded by the plant. She grew tomato, potato, maize and bean plants in cultures containing ^{32}P and at intervals subjected the leaves to artificial rain and analysed the rain water collected for ^{32}P.

Assuming that the ratio of the number of colonies developing on agar to the actual number of colonies on the leaf surface remains constant, then, if the presence of *Sporobolomyces* is determined by a supply on the leaf surface of nutrients from within the host, it is necessary to make further assumptions. As the number of *Sporobolomyces* colonies per cm.^{2} of leaf blade only increases after the leaf has lived half its life, either the nutrients are only then enough to support *Sporobolomyces* or some essential nutrient is only then liberated, perhaps because of approaching senescence. That the change is associated with senescence, which progresses from the distal end of cereal leaves, is suggested by the observation that there are generally more colonies per cm.^{2} at the distal end of each leaf than at the proximal end. Before this stage, the colonies are scattered at random, although amino-acids, favourable for the growth of some fungi are guttated from the distal hydathode (Greenhill & Chibnall, 1934; Coleman, 1954).

On the other hand, if the ratio of the number of colonies developing on agar to the actual colony number on the leaf surface does not remain constant, the absence of *Sporobolomyces* on agar from leaves sampled before the 50 % stage in their lives may reflect the physiological state of the fungus. There may be a long lag period between the establishment of a colony and the start of spore production. To fit the data the lag must vary with the season and be in accord with the changes in the life-span of the leaves. Thus, during winter, when the leaves have a life-span of 110 days, the lag period is approximately 55 days and in the spring and summer when the life-span is 80 days the lag is about 40 days. This explanation of the successive changes in colony number seems improbable, for at the temperatures prevailing during the summer Buller found the time from spore germination to subsequent spore production to be only 24 hr.

Discussion of the effect of moisture has so far centred mainly on spore dispersal, but the transference of substances from the leaf to its surface may also be accelerated in atmospheres approaching saturation. For simplicity, I have assumed that the colony number is increased only by new colonies formed by germinating spores, but old many-celled colonies may also disintegrate and each of its parts then develop into a separate colony.

Other fungi similar to *Sporobolomyces* have been reported in large numbers on and around rust infections, viz. *Sporidiobolus johnsonii* Nyland associated with *Phragmidium rubi-idaei* infections of *Rubus idaeus* (Nyland, 1949) and *Itersonilia perplexans* Derx with *Puccinia malvacearum* infections of *Althaea rosea* (Derx, 1948). In this study colonies of *Sporobolomyces* were not found congregated about pustules of powdery mildew, *Erysiphe graminis*. This may reflect the fact that the morphology of rust and mildew pustules differs. Rust fungi rupture the epidermis and broken cells are exposed, whereas mildew fungi only penetrate the epidermis by fine haustoria and do not expose broken cells.

It seems probable that *Sporobolomyces* has little effect on the development of leaf parasites, for leaves that were heavily infected with *Erysiphe graminis* were as heavily contaminated with *Sporobolomyces* as leaves free from mildew. There was some indication that *Sporobolomyces* and *Tilletiopsis* compete on barley leaves.

No observations were made which suggest that the presence of *Sporobolomyces* is either harmful or beneficial to the host. The presence of a highly developed non-parasitic flora near to living roots, the rhizosphere, has for long been recognized, but the recognition of a comparable flora of leaf surfaces is new. *Sporobolomyces*, *Tilletiopsis* and *Bullera* inhabit this environment, 'the phyllosphere', but they are probably only a fraction of the total flora because the isolation technique used selects in favour of fungi whose spores are forcibly discharged and can germinate and develop on agar.

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(*Accepted for publication* 11 *November* 1954)

Trans. Brit. mycol. Soc. **38** (3), 240–242 (1955).

SOME SEED-BORNE FUNGI FROM PAKISTAN

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This paper lists eighteen species of fungi isolated by the plating method from seeds collected from Lahore (West Pakistan) and its vicinity. The list includes *Melanospora zamiae*, *M. theleboloides*, *Nigrospora sphaerica*, *Epicoccum nigrum* and *Gymnoascus bourquelotii*.

Prof. Muskett (1950), in the course of his Presidential address to the British Mycological Society, remarked: 'The value of the study of seed-borne fungi as an aid to systematic mycology is something which should not be overlooked. An impetus to more intensive taxonomic studies is urgently required and the fungus flora of seeds offers scope to those who would select this field for their activity.' In order to make a study of the fungus flora found associated with seeds in the Panjab, samples of seeds collected locally or raised at the Panjab Agricultural Research Institute, Lyallpur, were examined by the plating method (Muskett & Malone, 1941; Groves & Skolko, 1944) between May 1953 and March 1954. The seeds were surface-sterilized after the method of Groves & Skolko before being plated out on agar, and the fungi which appeared on agar plates were isolated on 2% malt-extract agar slants. Permanent herbarium specimens were prepared in the manner described by Groves & Skolko and added to the Mycological collection of the Panjab University Herbarium. Of the fungi isolated, those which have been identified are listed below.

The species of *Chaetomium* were identified by Dr A. J. Skolko, species of *Curvularia* and *Nigrospora* by Dr J. W. Groves, *Melanospora* spp. by Dr E. Müller, *Fusarium* sp. by Dr W. L. Gordon and species of *Helminthosporium* and *Alternaria* by Dr S. P. Wiltshire. Our sincere thanks are due to these mycologists for their valuable help.

GYMNOASCUS BOURQUELOTII Boud.

No. 12023; isolated from cotton seeds.

CHAETOMIUM GLOBOSUM Kze.

No. 12021; isolated from seeds of *Pastinaca sativa* L. (According to Skolko & Groves (1953), this is the commonest species of *Chaetomium* isolated from seeds.)

CHAETOMIUM INDICUM Corda

No. 12018; isolated from seeds of *Spinacea oleracea* L.

Chaetomium brasiliense Bat. & Pont?

No. 12019; isolated from seeds of *Spinacea oleracea* L. Dr J. W. Groves remarked about this isolate: 'Not quite typical but seems close to this species. The spores are egg-shaped, the hairs are very rough but not coiled.'

Chaetomium aureum Chivers

No. 12020; isolated from cotton seeds.

Chaetomium gangligerum Ames

No. 12022; isolated from seeds of *Verbena* sp. and *Cheiranthus cheiri* L.

Melanospora zamiae Corda

No. 12024; isolated from cotton seeds. The fungus was grown on malt-extract agar, prune agar, pea agar and corn-meal agar, but it did not fruit on any of these culture media. The perithecia were obtained only when sterilized cotton seeds were added to the medium.

Melanospora theleboloides (Fuckel) Wint.

No. 12013; isolated from seeds of *Spinacea oleracea* L. and *Beta vulgaris* L.

Nigrospora sphaerica (Sacc.) Mason

No. 12017; isolated from seeds of *Helichrysum* sp.

Curvularia spicifera (Bain.) Boed.

No. 12005; isolated from seeds of *Daucus carota* L. and *Setaria glauca* Beauv.

Curvularia lunata (Wakker) Boed.

No. 12011; isolated from seeds of *Spinacea oleracea* L.

Curvularia trifolii (Kauffm.) Boed.

No. 12009; isolated from seeds of *Datura alba* Nees.

Curvularia penniseti (Mitra) Boed.?

No. 12003; isolated from seeds of *Daucus carota* L.

About this isolate Dr Groves wrote: 'I have never seen authentic material of this species but the spores seem to fit the description closer than any other.' The spores are 3-septate, ventricose-fusiform, olivaceous black and measure 36–43 × 12–16 μ.

Helminthosporium sativum Pammel

No. 12004; isolated from seeds of *Bombax malabaricum* DC.

ALTERNARIA TENUIS auct. sensu Wiltshire

No. 12014; this was the most commonly encountered species and was isolated from seeds of *Brassica campestris* L., *B. nigra* Koch, *Trigonella foenum-graecum* L., *Beta vulgaris* L., *Daucus carota* L., *Amaranthus viridis* L., *Allium cepa* L., *Triticum vulgare* Vill., *Avena sativa* L., *Pennisetum typhoideum* Rich., *Setaria italica* Beauv.

STEMPHYLIUM CONSORTIALE (v. Thüm.) Groves & Skolko

No. 12025; isolated from seeds of *Beta vulgaris* L. This species has been reported to occur on a wide variety of seeds including *B. vulgaris* L. (Groves & Skolko, 1944).

FUSARIUM SEMITECTUM Berk. & Rav.

No. 12015; isolated from seeds of *Bombax malabaricum* DC.

EPICOCCUM NIGRUM Link

No. 12016; isolated from seeds of *Poa annua* L.

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(*Accepted for publication* 6 *December* 1954)

Trans. Brit. mycol. Soc. **38** (3), 243–246 (1955).

THE PREVALENCE OF TWO SPECIES OF *CERCOSPORA* ON GROUNDNUTS

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(With 2 Text-figures)

In studies of the incidence, effects and control of *Cercospora* leafspots of groundnuts (*Arachis hypogaea* L.) in Tanganyika, observations were made of the relative prevalence of the two species involved. It was found that, although *Cercospora personata* appeared later in the season, its rate of build-up was so much more rapid than that of *C. arachidicola* that it became the major factor in the severe epidemic.

Groundnuts (*Arachis hypogaea* L.) in all parts of the world are commonly attacked by leafspots caused by *Cercospora personata* (Berk. & Curt.) Ell. & Everh. and by *C. arachidicola* Hori; very often both species occur in one area and can be found on the same plant (Commonwealth Mycological Institute, 1948). The perfect stages of these two fungi (*Mycosphaerella berkeleyii* W. A. Jenk. and *M. arachidicola* W. A. Jenk., respectively) are rarely found, and have not yet been recorded in East Africa.

It is apparent from references in the literature that in most areas where both species are found together, *Cercospora personata* is the major cause of loss. In Casamance, Chevaugeon (1952) records that, on a count on the variety ‘M’Bambey 28–206’ in the middle of the season, only 1·2 % of the leafspot lesions were caused by *C. arachidicola*. In several areas, such as Indonesia (van Hoof, 1950), *C. arachidicola* was only found when detailed investigations of the incidence of *C. personata* were made. However, in America, Woodroof (1933) reported that whereas formerly all leafspots had been attributed to *C. personata*, both species could cause destructive epidemics, and it seems that *C. arachidicola* is the major pathogen in Georgia (Woodroof, Cole & Hunter, 1944).

At Nachingwea, in the Southern Province of Tanganyika, both species are found on the groundnut crop, and the complex has caused heavy losses in yield (Hemingway, 1954). A report by Higgins (1935) states that resistance to the two species appears to be inherited independently, selections very resistant to one being often highly susceptible to the other. Because of this possibility, a number of observations of the relative prevalence of the two species was made during studies of the incidence and effects of the leafspots, and of the resistance of certain varieties to them.

A preliminary comparison of the incidence of the two species was made during the 1953 season on the variety ‘Natal Common’. Samples of twenty-five plants were taken at random at intervals and all the lesions classified and counted. After 63 days the numbers of lesions rose sharply as the epidemic increased, and classification was made thereafter on subsamples of leaves of twenty-five plants, including about 1000 lesions. The identification of the species causing the lesion is quite straightforward: after 3 days in a damp chamber lesions caused by *C. personata* bear, on the underside of the leaf, a dense dark brown to black stroma of conidiophores and conidia, visible easily to the naked eye, whereas those caused by *C. arachidicola* have no such stroma visible. The percentages of lesions caused by *C. personata* given in Table 1 show the increasing preponderance of this species as the season advanced.

Table 1. *Percentage of the total lesions caused by* Cercospora personata *on groundnut variety 'Natal Common' at Nachingwea in* 1953

Age of planting (days)	Percentage of lesions
37	—
40	—
51	—
56	8
58	18
61	31
63	29
68	39
76	45
85	56
91	72
99	93

During the 1954 season, samples of lesions were taken at frequent intervals (2–4 days) from two varieties, 'Kanyoma' and 'Mwitunde', growing in adjacent fields. Until the plantings were 85 days old the samples consisted of twenty-five plants taken at random, on which all the lesions were counted. After 85 days, with the increasing epidemic, the number of lesions per plant became uncountable and thereafter until harvest the lesions were classified and counted on samples of leaves taken by pulling off handfuls at random. These samples included 1000–1500 lesions. The graphs of the percentages of the lesions caused by *C. personata* for the two varieties are shown in Fig. 1. Between 70 and 100 days in age of planting the percentages increased sharply from approximately 20 to 90 %. The pronounced 'steps' in the graphs between the ages of 70 and 80 days (i.e. between 10 and 20 March) are due to the lower infection rates in the dry conditions of early March at Nachingwea in 1954.

These 'steps' can also be seen in Fig. 2, which compares the logarithmic increase of incidence of the two species of *Cercospora* on the variety 'Kanyoma'. From the straight-line parts of the graphs, it is found that a tenfold increase in incidence for *C. arachidicola* takes 23 days, for *C. personata* only 7 days.

This higher rate of spread is undoubtedly caused by the greater spore production of *C. personata*, in which the visible stromatal mass covers the whole of the lower surface of the lesion, whereas the conidiophore tufts of *C. arachidicola* are scantily distributed and can only be found with some magnification. There is no difference between the two species in the incubation periods from infection to sporulation of the new lesion, but the

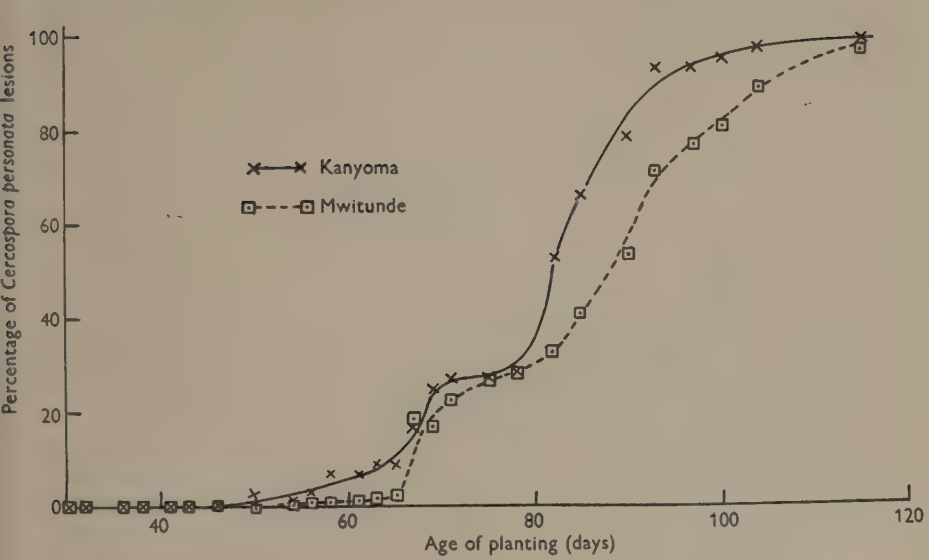

Fig. 1. Increasing preponderance of *C. personata* on two varieties of groundnut at Nachingwea, 1954.

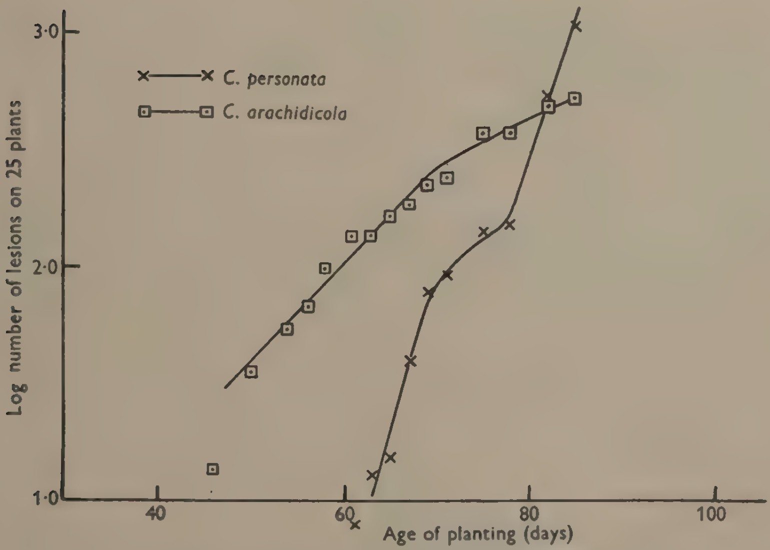

Fig. 2. Comparison of rates of increase of incidence of both species of *Cercospora* on variety 'Kanyoma' at Nachingwea, 1954.

C. arachidicola lesions usually grow to a larger size, and do not induce such rapid defoliation, so that they are able to maintain sporulation over a longer period (Table 2).

Table 2. *Area of lesions and rate of defoliation of the variety 'Natal Common' infected by* Cercospora *spp.*

Species	Mean area of lesions (in sample of 120 measured) ($\times \frac{1}{1000}$ in.^{2})	Mean time from infection to leaflet fall (days)
C. personata	10·57	19
C. arachidicola	16·29	23

In the counts of 'Kanyoma' and 'Mwitunde', lesions of *C. arachidicola* were first recorded on 24 January but *C. personata* was not found until 18 February. In the previous season the dates were 7 and 23 February, respectively. Thus, despite the earlier start made by *C. arachidicola*, it is the rapid build-up to heavy epidemic incidence of *C. personata* which causes extensive defoliation of the crop and associated losses in yield.

These studies were made on production fields of Litendanai Farm, Nachingwea, under the direction of Dr A. C. Evans (Experimental and Research Officer, O.F.C.).

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(*Accepted for publication 4 January* 1955)

Trans. Brit. mycol. Soc. **38** (3), 247–282 (1955).

THE GENUS *MONOBLEPHARIS*

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(With Plates 9–13 and 17 Text-figures)

An account is given of the genus *Monoblepharis*, all the previously known species of the genus, together with two new species and two new varieties having been collected and studied, i.e. *M. sphaerica* Cornu, *M. polymorpha* Cornu, *M. insignis* Thaxter (var. *insignis* and var. *minor* var.nov.), *M. fasciculata* Thaxter (var. *fasciculata* and var. *magna* var.nov.), *M. macrandra* (Lagerh.) Woronin (var. *macrandra* and var. *laevis* Sparrow), *M. bullata* sp.nov., *M. hypogyna* sp.nov.

M. insignis and *M. fasciculata* have not been described since their original description from America in 1895. The essentials of Thaxter's descriptions are corroborated, but new information is added and the scope of *M. fasciculata* is extended. *M. sphaerica* also has not been described since its original brief description by Cornu in 1871. This species is fully described, and recorded for Great Britain, for the first time. It is shown that Cornu's species is quite distinct from *M. sphaerica* Cornu emend. Woronin and a new species *M. hypogyna* is erected to include the latter.

Knowledge of the life history is extended, and information on the occurrence of the species in their natural habitats, together with conditions for growth in the laboratory are included. Suggestions are made to account for the infrequent collection of species of *Monoblepharis*.

The relationship of *Monoblepharis* to the other genera of the Monoblepharidales, i.e. *Monoblepharella* and *Gonapodya*, is discussed.

Type (or representative) material of all the forms described has been deposited in the Herbarium of the Commonwealth Mycological Institute, Kew.

INTRODUCTION

The genus *Monoblepharis* has been of special interest to investigators of the aquatic Phycomycetes since Cornu (1871) published his discovery of this new genus, which in contrast to all other known fungi possessed motile uniflagellate sperms. Three species, *M.* (*Gonapodya*) *prolifera*, *M. sphaerica* and *M. polymorpha*, were briefly characterized at that time, and in the following year the last two species were more fully described and illustrated in his *Monographie des Saprolegniées* (Cornu, 1872). Subsequent records are summarized in Table 1.

During the course of an investigation made in the vicinity of Cardiff, South Wales, all the known species and the one known variety were found, together with a new species, *M. bullata*, and two new varieties, *M. insignis* var. *minor* and *M. fasciculata* var. *magna*.

M. regignens Lagerheim and *M. ovigera* Lagerheim, which are known in the sporangial state only, are omitted from this account of the genus in view of the similarity of these imperfectly known species to species of the more recently described genus *Monoblepharella* Sparrow. This similarity has been the subject of comments by Sparrow (1940), Shanor (1942) and Springer (1945).

Table 1. *Summary of existing records and distribution of the genus* Monoblepharis

Author	Date	Country	*M. sphaerica*	*M. polymorpha*	*M. hypogyna*	*M. macrandra*		*M. insignis*		*M. fasciculata*		*M. bullata*
						var. *macrandra*	var. *laveis*	var. *insignis*	var. *minor*	var. *fasciculata*	var. *magna*	
Cornu	1871 1872	France	X	X	—	X*	—	—	—	—	—	—
Thaxter	1895 1903	N. America	—	X and X†	—	—	—	X	—	X	—	—
Lagerheim	1899	Sweden	—	X†	—	X‡	—	—	—	—	—	—
Minden	1902	Germany	—	X	—	—	—	—	—	—	—	—
Woronin	1904	Finland	—	X	X§	X	—	—	—	—	—	—
Petersen	1910	Denmark	—	X†	—	X‡	—	—	—	—	—	—
Tiesenhausen	1912	Switzerland	—	X	—	X	—	—	—	—	—	—
Minden	1915	Germany	—	X	—	X	—	—	—	—	—	—
Wettstein	1921	Austria	—	X	—	X	—	—	—	—	—	—
Laibach	1926	Germany	—	X	X§	X	—	—	—	—	—	—
Apinis	1929	Latvia	—	X and X†	X§	—	—	—	—	—	—	—
Barnes & Melville	1932	England	—	—	(X)§	(X)*	—	—	—	—	—	—
Sparrow Jr.	1933	N. America	—	—	X§	X	X	—	—	—	—	—
Sparrow Jr.	1933 1936	England	—	—	(X)§	(X)	(X)	—	—	—	—	—
Lund	1934	Denmark	—	X and X‡	X§	—	—	—	—	—	—	—
Mathews	1935	N. America	.	X	.	.	.	.	.	.	.	.
Indoh	1935 1937	Japan	—	X	—	—	—	—	—	—	—	—
Thomas [Perrott]	1939	South Wales	—	(X) and (X†)	(X)§	(X)	—	(X)	—	(X)	—	—
Beneke	1948	N. America	—	X	X	X	X	—	—	—	—	—
Thomas Perrott	This investigation	South Wales	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
		Cheshire	.	.	(X)	(X)	(X)	(X)	.	.	(X)	.
		N. Staffs	.	.	.	(X)	(X)	.	.	.	.	.

X = record; (X) = British record; — = no record.

* As *M. polymorpha*.

‡ As a variety of *M. polymorpha*.

† As *M. brachyandra*.

§ As *M. sphaerica* Cornu emend. Woronin.

Methods of collection and subsequent treatment of material

All the species of *Monoblepharis* have been found growing as saprophytes upon dead, entirely submerged twigs in fresh water, and after a preliminary investigation of a large number of fresh-water habitats, three localities in the vicinity of Cardiff, South Wales, were selected as collecting stations:

(1) A series of seven ponds situated in low-lying meadows on Triassic marls at Dinas Powis, 5 miles west of Cardiff.

(2) Drainage channels at St Mellons, 3 miles east of Cardiff. These channels are cut in the estuarine silt of this area to drain its low-lying meadows.

(3) A large pool named Pwll Diwaelod, at Castleton, 7 miles east of Cardiff, situated in meadowland on Old Red Sandstone marl with an overlying drift of glacial sands and clays.

All collecting stations were overhung by trees, and monthly collections of twigs were made over a period of 3 years. Those twigs which seem to provide the most suitable substrate for *Monoblepharis* were waterlogged and not decorticated. *Monoblepharis* has not been obtained from collections made in places where the water is more than a foot deep, the most suitable places being the shallow parts of ponds with a fresh water supply and shallow drainage channels. It is important that the water should be relatively free from silt in suspension, since the fungus seems mostly to invade the twigs through their lenticels. Hence those stations where the water is almost still, and free from frequent visits by cattle are the most, favourable. Light seems to have little effect upon the growth of *Monoblepharis* as it has been found growing in water with a surface growth of *Lemna minor* L. and *Ranunculus aquatilis* L. subsp. *peltatus* (Schrank) Syme emend., as well as in pools receiving a good measure of light.

Twigs collected during the summer months from dried-up ponds have produced growths of *Monoblepharis* after having been kept under suitable laboratory conditions. Survival during the unfavourable period is by means of the thick-walled, resistant oospores, which have been shown to germinate in the laboratory 2–4 months after collection.

The waters of the collecting stations were tested at monthly intervals for their hydrogen-ion concentration, and it was found that the fungus grew in waters which were 'neutrally alkaline' according to Lund's (1934) classification of aquatic habitats of phycomycetous flora, the pH values varying from pH 6·4 to 7·5.

Growths of *Monoblepharis* have been recorded by previous workers on the twigs of the following trees: *Betula* spp., Thaxter (1895), Lagerheim (1899), Sparrow (1932), Mathews (1935), Beneke (1948); *Fraxinus* spp., Laibach (1927), Lund (1934), Beneke (1948), Sparrow (1936); *Alnus* spp., Woronin (1904), Apinis (1929), Lund (1934); *Aesculus* sp., Sparrow (1936); *Prunus* sp., Indoh (1937); *Pinus* sp., Woronin (1904); *Picea* spp., Lund (1934), Tiesenhausen (1912); *Salix* sp., Beneke (1948); *Ulmus* sp., Beneke (1948); *Populus* sp., Beneke (1948); *Carya* sp., Beneke (1948).

During the course of this investigation, growths of *Monoblepharis* have been obtained from twigs of *Betula verrucosa* Ehrh., *Fraxinus excelsior* L., *Ulmus* sp., and *Quercus robur* L., the most profuse growths being obtained from the last.

Other types of substrate, such as submerged rose-hips (Sparrow, 1933), stems of *Phragmites communis* Trin. and *Equisetum fluviatile* L. (Lagerheim, 1899), dead insects (Cornu, 1871), and pieces of cuttlefish (Indoh, 1935), have also been described as producing growths of *Monoblepharis*. During this investigation collections of rose-hips, apples, and insect material from water did not produce growths of this fungus.

Suitable twigs were collected and placed between layers of wet paper in tins with well-fitting lids. The twigs were taken to the laboratory immediately after collection, and placed in sterile glass dishes, with loosely fitting glass-covers. These covers were supported on the rims of the dishes by small pieces of rubber tubing to allow air to enter. The dishes were filled with sterile distilled water to a depth sufficient to cover the twigs. Material examined as soon as it was brought in from the field only occasionally showed visible growths of *Monoblepharis*. Twigs collected during the spring and autumn months mostly produced vigorous indentifiable growths in 2–4 weeks after being brought into the laboratory. On the other hand, twigs collected during the summer and winter months produced identifiable growths only after 1–4 months, the length of time varying according to whether the substrate was collected at the end or near the beginning of the resting period. Under field conditions the germination of the oospores, which remain dormant during the winter months, usually takes place in March, April and May, and another set of oospores is formed on the newly formed mycelia. The summer months of June, July and August are a resting period for these spring-formed oospores, which germinate in September, October and November to form mycelia. These mycelia soon form oospores which remain dormant during December, January and February. Hence in any one year there are two periods of germination and vegetative growth, one in spring and another in autumn, with resting periods during the summer and winter months. All species of *Monoblepharis*, therefore, reach maximum vigour in this country during the colder months of spring and autumn, a fact which, together with their slow growth and consequently late appearance in gross culture, may account for their infrequent collection and supposed rarity.

When profuse growths of *Monoblepharis* are formed, the fungus is clearly visible as pale grey tufts of very delicate hyphae 1–2 mm. long, at the lenticels or at the broken ends of the twig. When the fungus is reproducing sexually, these tufts are yellowish brown, due to the presence of large numbers of golden brown oospores.

During this investigation the dishes of distilled water containing suitable twigs were placed in a cold room at a constant temperature of 3° C. and were brought into the laboratory only for examination. At 3° C. *Monoblepharis* grew vigorously and reproduced both by asexual and sexual means, although at collecting stations during the vernal period of germination and growth, water temperatures have been found to vary from 1 to 11° C.

By repeated washings with sterile distilled water, and the selection of isolated growths, it was possible to keep rough cultures growing upon the original substrate, fairly free from other organisms, for a considerable length of time. The constant low temperature appears to reduce the growth of other Phycomycetous fungi, such as members of the Saprolegniaceae, which tend to crowd out the slow-growing and more delicate *Monoblepharis* at laboratory temperatures (approximately 20° C.).

The species of *Monoblepharis* described here were studied from a large number of such rough cultures. The exogenous species have been obtained in pure culture on a natural sterile medium, but efforts to obtain vigorous identifiable growths on an artificial medium have not been successful. The main difficulties encountered were that the fungus produced only vegetative mycelia on agar, and that zoosporangia which seemed to be the only practicable starting-point for pure culture work have not been obtained in the endogenous species. The pure culture method which proved most successful was as follows:

(1) Material of *Monoblepharis* growing upon its natural substratum was left to develop in a mixture of 50% autoclaved pond water and 50% sterile distilled water.

(2) When sporangia were formed the material was transferred to fresh sterile distilled water, which causes the sporangia to dehisce. The dehiscing sporangia were kept under observation, and zoospores caught by means of a sterile Pasteur pipette.

(3) The zoospores were washed several times in sterile distilled water, and transferred with one drop of sterile water to a Petri dish of the following medium: $NaNO_3$, 2·0 g.; KCl, 0·5 g.; K_2HPO_4, 0·5 g.; $MgSO_4$, 0·5 g.; $Fe_2(SO_4)_3$, 0·01 g.; sucrose, 15 g.; peptone, 5·0 g.; Difco corn meal agar, 20·0 g.; sterile distilled water, 500 ml.; filtered autoclaved pond water, 500 ml. On this agar the fungus produced only vegetative mycelia.

(4) Small blocks of agar containing a portion of mycelium were transferred from the Petri dish to flasks containing a sterile liquid medium containing 25% of the amounts of minerals, sugar and peptone used for the agar to 500 ml. sterile distilled water and 500 ml. filtered autoclaved pond water. In this liquid sporangia developed.

(5) On the formation of sporangia the fungus was transferred to wide-mouthed litre flasks of sterile water (made up as above) for the discharge of the zoospores. To each of these flasks a small tomato was added, the surface of which was first sterilized by placing it in 95% alcohol and flaming. After addition to the flask its skin was pricked with a sterilized needle. On this natural medium the fungus produces vigorous growth with sexual reproductive organs in about 4 weeks.

THE LIFE HISTORY OF *MONOBLEPHARIS*

The zoospores of *Monoblepharis* produce a germ tube which divides to give rise (1) to the rhizoidal system which anchors the plant to the substrate; and (2) to the main body of the mycelium.

Once the fungus is established on its substrate under favourable conditions an abundant mycelial growth is produced. The hyphae of *Monoblepharis* are almost always recognizable when examined microscopically by their characteristic vacuolation. This is due to the protoplasmic contents forming a network of finely granular strands usually crossing the filament at right angles and enclosing vacuoles which are remarkably regular in size and form. Under changing environmental conditions, however, the protoplasm may temporarily assume a non-vacuolate homogenous structure.

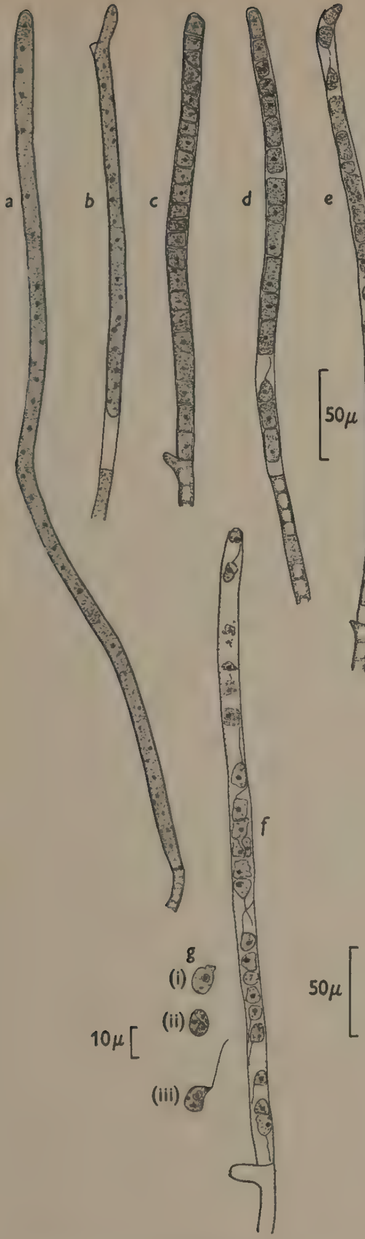
ASEXUAL REPRODUCTION

Under favourable conditions for growth asexual reproductive organs are formed. This was observed in *M. macrandra*, *M. polymorpha* and *M. hypogyna*, that is, in all those species having exogenous oospores.

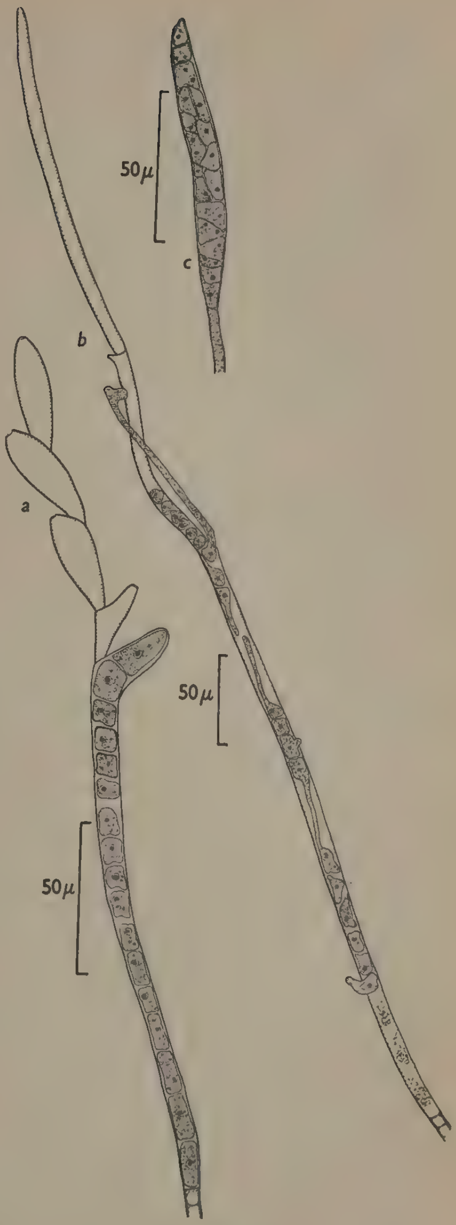
These species show little variation in their asexual reproductive organs, and differences between species relate mainly to size. The sporangia usually arise as terminal, slightly swollen, narrowly cylindrical portions of the hyphae. At first they possess homogeneous contents and many nuclei. Each sporangium is separated by a cross-wall from its sporangiophore, and as the cross-wall is laid down, the nuclei become more or less equidistant from one another (Text-fig. 1*a*, *b*). Cleavage planes in the protoplasm mostly appear at right angles to the long axis of the sporangium (Text-fig. 1*c*), although some may be parallel to it (Text-fig. 1*f*). Coincidental with protoplasmic cleavage there is a marked increase in the size of the individual nuclei (Text-fig. 1*d*), which now exhibit masses of dark-staining material round their edges. Laibach (1927) suggests that this material may be concerned with the formation of the flagellum. The spore initials are at first angular in outline, but gradually become pear-shaped (Text-fig. 1*c*–*f*). During this process all the protoplasm may not be used up in the formation of spores (Text-fig. 1*e*), small portions remaining within the sporangium after the discharge of the zoospores. A pore is formed at the apex of the sporangium through which the zoospores creep in an amoeboid fashion. Often a zoospore may remain adherent to the mouth of the sporangium by means of its flagellum, and may oscillate here for some little time before becoming free by a rapid succession of vigorous jerks. At this time the zoospore exhibits almost homogenous contents in which are embedded a few refractive granules (Text-fig. 1*f*). When the zoospore is free from the sporangium it either immediately darts away, or floats for a time, feebly moving its flagellum. The latter slowly increases its rate of vibration, however, and, trailing behind, propels the spore through the water.

Upon germination the nucleus of the zoospore divides, and further divisions take place as the hypha grows in length. Zoospores may often be found germinating within the sporangium (Text-fig. 2*b*). A cytological examination of the zoospores, using the method outlined by Cotner (1930), confirmed the very careful and full report by Sparrow (1933).

Occasionally sporangia are borne in a row at the tip of a hypha (Text-fig. 2*b*), or in association with series of sexual reproductive organs (Text-fig. 2*a*). The terminal sporangium may sometimes be shorter and wider than normal, the zoospores forming two rows inside instead of the usual one (Text-fig. 2*c*).



Text-fig. 1.



Text-fig. 2

Text-fig. 1. *M. macrandra* var. *macrandra*. Stages in sporangial development. *a*, terminal sporangium containing dense protoplasm and many nuclei. *b*, sporangium with contents moving out through exit tube before differentiation. *c*, division of sporangial contents. *d*, *e*, and *f*, progressive stages in zoospore formation. *g*, (i) zoospore beginning to germinate; (ii) a trinucleate mass of sporangial contents which will divide to form three zoospores; (iii) a zoospore which is ready to be liberated.

Text-fig. 2. Sporangia of *M. macrandra* var. *macrandra*. *a*, Hypha bearing at its tip a superimposed series of reproductive organs consisting of three empty oogonia, one empty antheridium, and a young intercalary sporangium containing zoospore initials. *b*, a hyphal tip bearing three superimposed sporangia (one sporangium shows zoospores germinating *in situ*). *c*, a terminal sporangium which is shorter and wider than is typical for sporangia in this species, zoospores forming two rows inside.

SEXUAL REPRODUCTION

The development of the sexual organs

In *M. polymorpha*, *M. insignis*, *M. fasciculata*, and *M. bullata*, the antheridia appear to be inserted on the oogonia (epigynous). A study of their development, however, indicates that the oogonium is formed beneath the terminal antheridium, and is an intercalary structure (Text-figs. 10, 17). In these species the antheridium originates from the terminal portion of a hypha, the contents of which are homogeneous, containing many nuclei (Text-fig. 17*c*). It becomes cut off from the rest of the hypha by a cross-wall, the nuclei become more or less equidistant from one another, and the differentiation of the antherozooids is initiated. The cleavage planes appear in most instances at right angles to the long axis of the antheridium, although some may appear parallel with it (Text-fig. 17*d*). The antherozooid initials are at first angular in outline, but gradually they become rounded (Text-figs. 17*d*, *e*, 6*a*, 7*h*). The antherozooids produced in a single antheridium vary from 4 to 8 in *M. insignis*, *M. fasciculata* and *M. bullata* (Text-figs. 3*b*, 7*i*, 10*b*), whereas in *M. polymorpha* there are from 5 to 12 (Text-fig. 17*d*, *h*). In shape, internal structure and flagellation they resemble the zoospore, but differ from the latter in their smaller size and more pronounced amoeboid movements. They move along the walls of the antheridium in an amoeboid fashion and escape through a small opening caused by the deliquescence of the tip.

As soon as the antheridium is cut off, the proximal portion of the hypha, just beneath the antheridium, gradually becomes distended and forms a lateral somewhat oblique projection (Text-figs. 7*h*, 10*a*, 17*d*). This lateral distention continues and a clavate body is formed, which is eventually separated from the hypha by a basal cross-wall (Text-fig. 10*b*–*d*). The antheridium has often begun to discharge its antherozooids before the formation of this oogonial septum (Text-figs. 3*b*, 7*h*). The oogonium thus delimited gradually becomes more rounded in shape, and the minute, evenly disposed oil droplets in the ooplasm combine to form large refractive globules (Text-fig. 17*e*), occupying a central position in the oogonium. The oogonial contents are then seen to move slightly away from the oogonial wall, and the oosphere or egg is defined (Text-figs. 13*c*, 14*b*, 5*a*, *b*). The oosphere is uninucleate, the nucleus ultimately attaining a terminal position in the oogonium, as shown by Lagerheim (1899) and Laibach (1927) for *M. polymorpha* and *M. macrandra*.

M. sphaerica, *M. hypogyna* and *M. macrandra* differ from the species previously described in the order of development of the sexual reproductive organs. In *M. sphaerica* and *M. hypogyna* the oogonium originates as the terminal portion of a hypha which is swollen with dense contents in which are embedded numerous oil globules (Text-fig. 13*a*). After the oogonium is cut off by a cross-wall, that portion of the hypha immediately beneath it forms a small lateral projection (Text-fig. 13*b*). Another transverse wall limits this portion of the hypha forming a hypogynous antheridium, and the differentiation of the antherozooids proceeds in the same manner as described for the epigynous species (Text-fig. 13*c*). The antherozooids escape through the tip of the slightly exserted projection which is immediately beneath the basal wall of the oogonium (Text-figs. 12, 13).

In *M. macrandra* the antheridia and oogonia develop in the same manner as described for other species of the genus, the difference here being that both antheridia and oogonia are borne terminally on different hyphal branches (Text-fig. 14). As growth and reproduction proceed, however, other oogonia and antheridia are formed beneath the terminal ones, producing superimposed linear series of either oogonia or antheridia. Later still the diclinous habit is lost and oogonia and antheridia are found together on the same hypha with either (1) both antheridia and oogonia terminal in position; (2) oogonia terminal and antheridia hypogynous; or (3) oogonia and antheridia terminal and intercalary in alternating groups (Text-fig. 16).

Fertilization and the maturation of the oospore

The process of fertilization is similar in all the species of the genus. It has been fully described and illustrated for *M. sphaerica* by Cornu (1872), for *M. polymorpha* by Cornu (1872) and Sparrow (1933), for *M. macrandra* (as *M. polymorpha*) by Barnes & Melville (1932), and for *M. hypogyna* (as *M. sphaerica*) by Woronin (1904); therefore it is not described in detail here.

When the oosphere has been formed and is ready for fertilization, the oogonium opens at its tip. An antherozooid, on approaching the apex of the oogonium, seems to become caught in a secretion of mucus from the oogonial tip, and is immediately engulfed, soon becoming indistinguishable from the egg (Text-fig. 5*b*). The flagellum of the antherozooid may protrude for a few minutes, but it too is finally absorbed.

A thin golden brown membrane begins to form around the zygote before the fusion of nuclei has taken place.

In endogenous species, *M. insignis*, *M. fasciculata*, *M. bullata*, and *M. sphaerica*, the oospore remains within the oogonium after fertilization and forms a thick golden brown exospore. The exospore remains smooth in *M. insignis*, *M. fasciculata* and their varieties, but becomes bullated in *M. sphaerica* and *M. bullata* (Text-figs. 3, 4, 6, 7, 11 and 12).

In the exogenous species, *M. hypogyna*, *M. macrandra* and *M. polymorpha*, the oospore begins to leave the oogonium through the opening at the tip, 3–5 min. after fertilization. The evacuation of the oogonium is gradual, the process taking at least 2 min. (Text-fig. 14*d*–*h*). Once outside the oogonium the oospore remains attached to the oogonial mouth, except in *M. macrandra* where the oospores tend to fall away from the oogonial mouth after a very short period of attachment. As Lagerheim (1899) and Laibach (1927) have observed, the male and female nuclei in these species do not immediately fuse on the union of the antherozooid and oosphere, but remain side by side until exospore formation has reached an advanced stage (i.e. when bullations are beginning to form in species which possess them). Fusion then takes place and the mature oospore is uninucleate. It is interesting to note that in *M. polymorpha* the oospore wall remains thin and smooth until the oospore has emerged from the oogonium. Once outside, the wall thickens and bullations begin to form (Text-fig. 17*f*–*h*). On the other hand, bullations are formed on the exospore while the oospore is still in the process of evacuating the oogonium in *M. macrandra* and *M. hypogyna* (Text-figs. 17*d*, 14*e*–*h*).

After a resting period, which coincides with frozen winter conditions or summer drought, the oospore germinates with the onset of favourable conditions for growth, producing a single hypha which branches to form a mycelium.

Parthenogenetic oospores

Unfertilized oogonia which develop parthenogenetically to form thick-walled oospores are often to be seen in: *M. macrandra* var. *macrandra* (Text-fig. 15*a*, *b*), *M. macrandra* var. *laevis* (Text-fig. 16), *M. polymorpha* (Text-fig. 15*c*–*h*), *M. hypogyna* (Text-fig. 13*g*), *M. bullata* (Text-fig. 11*b*, *d*), *M. insignis* var. *minor* (Text-fig. 7*f*, *k*), and *M. fasciculata* (Text-fig. 3*c*, *d*). Occasionally these oogonia are of normal size and shape (Text-fig. 16*a*), but usually they are larger than normal (Text-fig. 13*g*), or of an abnormal shape (Text-fig. 15*a*, *d*, *f*). As a rule these oogonia remain closed until after the oospore is formed. In species in which normal oogonia bear epigynous antheridia the abnormal oogonia do not bear antheridia (Text-figs. 15*a*, *f*, *h*, 11*b*, 3*d*). The parthenogenetic oospores usually have smooth walls in those species where normal oospores are bullated (Text-figs. 13*g*, 11*b*, *d*). Sometimes these parthenogenetic oospores are seen to divide, forming smaller spores before emergence from the oogonium (Text-figs. 11*c*, *d*, 3*d*).

TAXONOMY

Monoblepharis Cornu

Type species: *M. sphaerica* Cornu, *Bull. Soc. bot. Fr.* **18**, 58–59, 1871. On submerged plant and animal remains, France.

Syn. *Diblepharis* Lagerheim, *Bih. svensk. VetenskAkad. Handl.* **25**, 1–42, 1899.

Mycelium non-septate, branched or unbranched, colourless or with a slightly brownish tinge, attached by rhizoids to the substratum; hyphal contents disposed in a reticulate manner.

Zoosporangia usually terminal, occasionally arranged in rows formed in basipetal succession; narrowly cylindrical, cut off from the hyphae by cross-walls. Zoospores fully formed within the sporangium, escaping after the dissolution of its apex; posteriorly uniflagellate; producing a vegetative hypha on germination; often germinating *in situ*.

Oogonia terminal or intercalary, narrowly pyriform, spherical or cylindrical with a lateral projection or 'neck'; cut off from the adjacent hyphae by cross-walls; each oogonium containing a single oosphere and exhibiting a receptive papilla upon maturity.

Antheridia variable in shape, usually cylindrical, sometimes geniculate; variously placed; forming a small number of uniflagellate and amoeboid antherozooids which are slightly smaller than the zoospores; escaping from the antheridium by a terminal opening; maturing before the oogonium.

Fertilization taking place within the oogonium. Oospores remaining within the oogonium (endogenous) or emerging (exogenous); if the latter, usually remaining attached to the oogonial mouth, and developing thick golden brown walls which may be smooth or bullated when fusion is complete. Oospores upon germination producing a vegetative hypha.

The seven species (including six varieties) of *Monoblepharis* in which sexual reproduction occurs fall into two groups:

A. Subgenus. *Monoblepharis.*

Syn. *Eumonoblepharis* Lagerheim, *Bih. svensk. VetenskAkad. Handl.* **25**, 1–42, 1899.

Oospores always endogenous. Oogonia either spherical to subspherical or cylindrical with a lateral projection called the 'neck'. Antheridia variously borne. Zoosporangia not observed.

B. Subgenus. *Exoospora* Lagerheim, *Bih. svensk. VetenskAkad. Handl.* **25**, 1–42, 1899.

Oospores always exogenous. Oogonia pyriform. Antheridia variously borne. Zoosporangia cylindrical, usually terminal, occasionally in rows, producing uniflagellate zoospores.

Key to the species of Monoblepharis

A. Oospores endogenous subgen. **MONOBLEPHARIS**

I. Oogonia cylindrical with a lateral projection called the 'neck', antheridia epigynous, i.e. appearing to be inserted upon the oogonium.

(*a*) Oospores smooth.

(i) Sex organs arranged in fascicles. **M. fasciculata**
Oospores 21–25μ × 18μ. var. **fasciculata**
Oospores 28–32μ × 23μ. var. **magna**

(ii) Sex organs arranged in superimposed series or singly, at the tips of hyphae. **M. insignis**
Oospores 30–40μ × 23–32μ var. **insignis**
Oospores 23–25μ × 20–21μ. var. **minor**

(*b*) Oospores bullated.
Sex organs arranged in fascicles, superimposed series or singly at the tips of hyphae. **M. bullata**

II. Oogonia subspherical to spherical. Antheridia hypogynous, i.e. formed immediately below the oogonium.
Oospores bullated. **M. sphaerica**

B. Oospores exogenous. subgen. **EXOOSPORA**
Oogonia pyriform.

I. Antheridia hypogynous, i.e. formed immediately below the oogonium, scarcely exserted.
Oospores bullated. **M. hypogyna**

II. Antheridia occurring singly or in groups on separate branches from the oogonia at first; at later stages of development in groups with the oogonia, occasionally hypogynous, conspicuously exserted, oospores tending to fall away from the oogonial mouth. **M. macrandra**

(*a*) Oospores bullated. var. **macrandra**

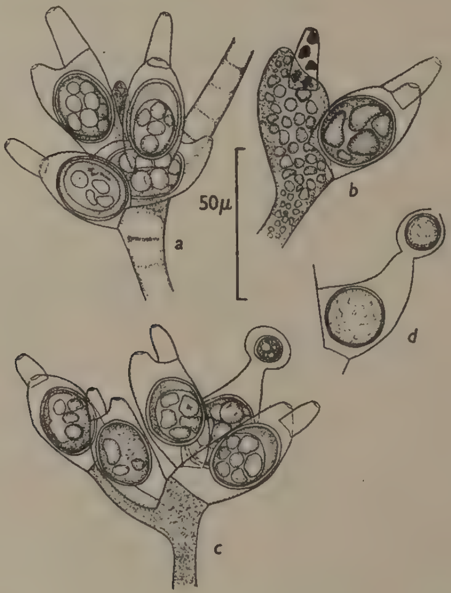
(*b*) Oospores smooth. var. **laevis**

III. Antheridia epigynous, i.e. appearing to be inserted on the oogonium, sometimes irregular at point of insertion of the antheridium.
Oospores bullated. **M. polymorpha**

Monoblepharis fasciculata Thaxter, *Bot. Gaz.* **20**, 433–440, 1895.

Monoblepharis fasciculata Thaxter var. **fasciculata** (Pl. 9; Text-fig. 3)

Mycelium well developed and composed of straight, rigid, cylindrical, slightly brownish, hyaline, vacuolated hyphae, 1–2 mm. long, attached to the substratum by branched rhizoids. Hyphae are simple or occasionally sympodially branched, the diameter varying from 9 to 12·5μ in the basal and terminal portions to 5–7μ in the intermediate portions.



Text-fig. 3. *M. fasciculata* var. *fasciculata*. *a*, a fascicle of mature oogonia and their epigynous antheridia, borne at the tip of a sympodially branched hypha. *b*, hyphal tip showing one mature oogonium containing a single smooth-walled oospore and bearing an empty epigynous antheridium, together with a young oogonium, its contents rich in oil globules, bearing an epigynous antheridium containing five antherozooids. *c*, a sympodially branched hypha bearing a fascicle of mature reproductive organs. One of the oogonia does not bear an epigynous antheridium, and its 'neck' has produced a closed spherical chamber at the tip. This chamber contains a rounded spore which has been cut off from the oospore. *d*, a single oogonium (removed from a fascicle) the 'neck' of which has swollen terminally into a spherical chamber containing a large spore which has been cut off from the oospore in the main portion of the oogonium. The mouth of this chamber has opened to allow the spore to emerge.

Oogonia cylindrical with a straight or slightly curved lateral projection called the 'neck', which is always shorter than the antheridium and which usually opens at its tip when the oosphere is formed. The oogonia vary in length from 23 to 46μ (majority 23–39μ); diameter 15–27μ (majority 20–21μ). They are arranged singly or in fascicles of 2–8 (majority 3–5), at the tips of the hyphae (Text-fig. 3*a*, *c*).

Antheridia cylindrical, tapering slightly, narrow, straight, not divergent, and longer than the neck of the oogonium. They vary from 10·5 to 27 μ in length (majority 12–18 μ) by 5–12·5 μ in diameter (majority 7–9 μ); always present on normal oogonia, and borne upon the terminal wall (epigynous), at one side of the neck. A single antheridium usually contains 5–8 amoeboid and uniflagellate *antherozooids* which are 3 μ in diameter. Protandry is wellmarked (Text-fig. 3).

Each oogonium contains a single oospore which matures and germinates *in situ* (endogenous). The *oospores* vary in shape from subspherical to widely ellipsoid, are golden brown in colour, and have smooth walls; length 17–27 μ (majority 21–25 μ), diameter 13–25 μ (majority 18 μ).

On submerged twigs of *Quercus robur L.* at St Mellons and Dinas Powis, South Wales.

M. fasciculata var. *fasciculata* has not been described since the brief original description by Thaxter (1895) which contains few measurements, viz. diameter of hypha 6 μ, diameter of antherozooid 3 μ, and oospore 22 × 18 μ, and was apparently based on only two collections, in which material of *M. fasciculata* was not abundant. Although many collections have been examined, one stage described by Thaxter cannot be confirmed. He describes zoosporangia which are like oogonia, bearing antheridia; the zoospores biciliate, about 5–6 μ in diameter. It has been suggested by Sparrow (1933), who has examined Thaxter's specimens at the Farlow Herbarium, that the biciliate bodies which Thaxter described as zoospores were extraneous, parasitic organisms that had forced their way into the oogonium to feed on its disintegrating contents. During this investigation oogonia were frequently seen in which the contents break down and leave the oogonium. This disintegration of oogonial contents seems to occur where the oosphere is not fertilized. The resemblance between the emerging contents of these oogonia and zoospores has often been noted. Parasites have not been observed in the oogonia of this species, although they have sometimes been seen invading the hyphae.

Occasionally abnormal oogonia, which are not noted by Thaxter, are found among the normal oogonia of a fascicle. These are oogonia with elongated necks which terminate in a spherical chamber, 12–16 μ in diameter. They do not bear an antheridium, and do not open until after the single oosphere has matured, which suggests a parthenogenetic development. The oospores thus formed are seen to produce by constriction a small, spherical, smooth-walled spore, 9–12 μ in diameter, which passes into the chamber at the tip of the oogonium, from which it is set free. (Text-fig. 3*c*, *d*). Similar oogonia have been observed in *M. insignis* var. *minor* and *M. bullata*, while others which differ from these only in being without the terminal spherical chamber have been found in *M. macrandra* and *M. polymorpha*. Lagerheim (1899) figured the latter type for *M. polymorpha* (syn. *M. brachyandra* Lagerheim), and described them as oogonia containing parthenogenetic oospores.

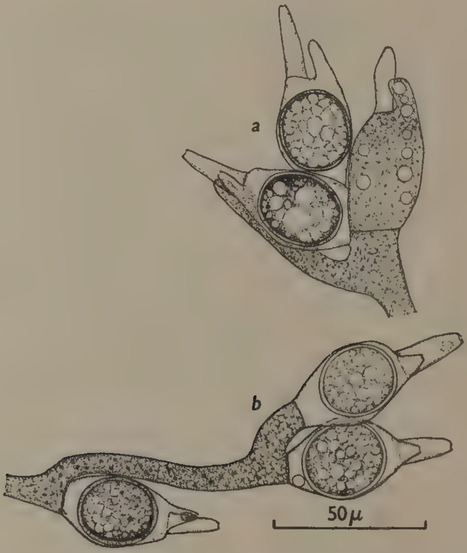
As a result of this investigation, the range of *M. fasciculata* var. *fasciculata* has been increased to include oospores 17–27 μ in length (majority 21–25 μ), and 13–25 μ in diameter (majority 18 μ); and hyphae 5–12·5 μ in diameter. These limits were based on 500 measurements of oospores taken from samples of material collected at different times of the year, from different collecting stations, and on different substrates. By comparison of data it was seen that environmental changes did not cause differences in the frequency of occurrence of oospore sizes or in the limits of variation.

Monoblepharis fasciculata Thaxter var. **magna** var.nov. (Text-fig. 4)

Mycelium hyphis rectis, raro ramosis, 1–2 mm. longitudine, 7–12·5 μ diam. (maior pars 7–10·5μ).

Oogonia varia longitudine, 32–53μ (maior pars 39–50μ), 21–36μ diam. (maior pars 25μ).

Antheridia varia longitudine 14–29μ (maior pars 21–25μ), 5–11μ diam. (maior pars 7–9μ, contractione ad 6–7μ). Antherozooida 6–7 numero, 4–5μ diam.



Text-fig. 4. *M. fasciculata* var. *magna* var.nov. *a*, tip of a sympodially branched hypha, bearing three oogonia arranged in a fascicle—typical arrangement. Each oogonium bears an empty antheridium. Two of the oogonia contain a single smooth-walled endogenous oospore. In the remaining oogonium the oosphere has not yet formed, and it is filled with dense protoplasm rich in oil globules. *b*, a hypha with the sexual reproductive organs arranged singly at the tips of the sympodially branched hypha. The youngest and terminal antheridium contains one antherozooid, the others having emerged. An antherozooid is also to be seen inside the neck of the oldest oogonium, which already contains an oospore.

Oosporae luteae, leves, varia forma, vel sphaericae vel ellipticae, vel oblongatae: ongitudine 24–36μ (maior pars 28–32μ).

Saprophytes est in ramulis submersis *Quercus roboris*, Dinas Powis, Glamorgan, South Wales, October 1938.

Mycelium well developed, consisting of straight, rigid, cylindrical, vacuolated hyphae, which are often pale brown, especially in the basal portions, and hyaline near the tips; rarely branched, except near the tip, 1–2 mm. long by 7–12·5μ in diameter (average 7–10·5μ); attached to the substratum by branching rhizoids.

Oogonia arranged singly or in fascicles at the tips of hyphae, cylindrical, contain a single oosphere, and open by a tube termed the 'neck' which is usually shorter than the antheridium and curved towards it. Occasionally this tube is as long as the antheridium. Oogonia vary in length from 32 to 54μ (majority 39–50μ); diameter 21–36μ (majority 25μ).

Antheridia long, cylindrical, and tapering at the tip, rarely subconical, usually straight, but occasionally slightly curved towards the neck of the oogonium; length 14–29μ (majority 21–25μ); diameter 5–11μ (majority 7–9μ tapering to 6–7μ); always present, borne on the oogonium (epigynous) to one side of the neck, which they always exceed in length. *Antherozooids* 4–5μ in diameter, amoeboid and uniciliate; usually 6 or 7 in each antheridium. Protandry is well marked.

Each oogonium contains a single oospore which matures and germinates *in situ* (endogenous). *Oospores* pale brown in colour, walls smooth, spherical, widely ellipsoid and cylindrical; length 24–36μ (majority 28–32μ), diameter 17–29μ (majority 23μ).

On submerged twigs of *Quercus robur* L. at St Mellons and Dinas Powis, Glamorgan, South Wales. Subsequently collected from ponds at Styal, Cheshire.

This variety resembles *M. fasciculata* var. *fasciculata* in every detail except for its larger size. *M. fasciculata* var. *magna* was not so abundant in these collections as the var. *fasciculata*. It does not seem likely that this large variety is produced as a result of growth under a particularly favourable set of environmental conditions, as it has been found growing upon the same twigs as var. *fasciculata*.

The smooth-walled oospores of *M. fasciculata* are always endogenous. Laibach (1927) regarded the endogenous position of the oospores of *M. fasciculata* and *M. insignis* as being due to poor environmental conditions. Material of *M. fasciculata* was collected from different collecting stations under a great variety of environmental conditions, and exogenous oospores have never been observed. Also, these endogenous species were found growing on the same twig, with *M. macrandra*, *M. polymorpha* and *M. hypogyna*, in which all fertilized and mature oospores were exogenous in position.

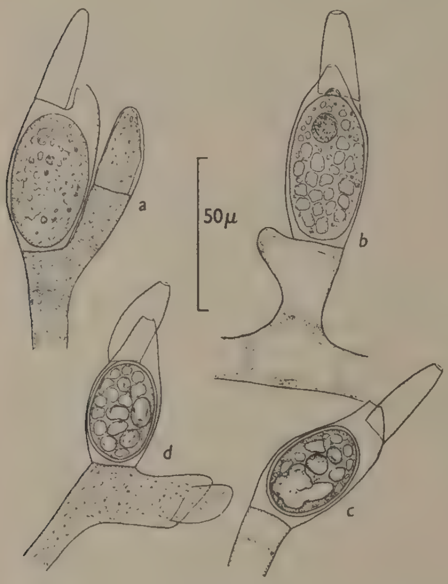
Monoblepharis insignis Thaxter, *Bot. Gaz.* **20**, 433–440, 1895

Monoblepharis insignis Thaxter var. **insignis** (Text-figs. 5, 6)

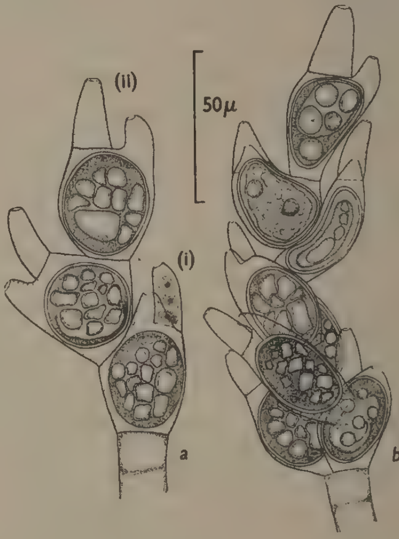
Mycelium composed of straight, rigid, hyaline hyphae 1·5–2 mm. long, sometimes with a brownish tinge, rarely branched, attached to the substratum by rhizoids. Diameter 11–16μ in the basal portions and at the tips, where the reproductive organs are borne, 9–11·5μ in the intermediate portions. Vacuolations of the hyphal protoplasm are often not so well marked as in the smaller species of the genus.

Oogonia at the tips of the hyphae, arranged either singly (Text-fig. 5*a–c*) or in series of 2–8, formed in basipetal succession (Text-fig. 6); occasionally intercalary; length 39–54μ, diameter 25–35μ. Cylindrical or irregular in shape, with a prominent lateral neck, which may be straight or slightly curved. The neck is often almost as long as the antheridium, although occasionally very short. The oogonium opens at the tip after the oosphere is formed.

Antheridia broad, subconical, occasionally subcylindrical, straight or slightly curved (Text-fig. 6*a*), nearly always present, borne upon the terminal wall of the oogonium (epigynous), to one side of the neck; length 20–27μ (majority 21–25μ); basal diameter 9–13μ (majority 12μ), much variation in the diameter of the antheridium being seen in the same series of sexual reproductive organs. Each antheridium contains 4–6 amoeboid, uniflagellate *antherozooids* 5–6μ by 3–5μ. Protandry is well marked.



Text-fig. 5.



Text-fig. 6.

Text-fig. 5. *M. insignis* var. *insignis*. *a*, a sympodially branched hyphal tip bearing an oogonium which contains an oosphere and an empty epigynous antheridium. The young branch of the hypha has cut off a portion at its tip which will become an antheridium. *b*, a short lateral branch bearing a single oogonium which contains an oosphere which is being fertilized. The antherozooid is to be seen entering the oosphere. The epigynous antheridium is empty. *c*, a terminal oogonium bearing a dehisced epigynous antheridium, and containing a thick-walled oospore. *d*, a hypha bearing at its tip a mature oogonium which contains an endogenous oospore rich in oil-globules, and an epigynous antheridium. Beneath this set of reproductive organs the hypha has formed a young antheridium, and is in the process of forming an oogonium.

Text-fig. 6. *M. insignis* var. *insignis*. *a*, a short series of superimposed sexual reproductive organs, formed in basipetal succession. *b*, a series of seven sets of mature reproductive organs. All the epigynous antheridia have shed their antherozooids, and each oogonium contains a mature oospore.

Each *oogonium* contains a single oospore, which matures and germinates within the oogonium (endogenous). *Oospores* widely ellipsoid, spherical and irregular (Text-fig. 6*b*); walls smooth and golden brown in colour, length 30–40μ, diameter 23–32μ. The contents of some oogonia are seen to break down and leave the oogonium, this disintegration of oogonial contents seeming to occur where the oosphere has not been fertilized.

On submerged twigs of *Quercus robur* L., St Mellons and Dinas Powis, South Wales, and Styal, Cheshire.

The zoosporangia described by Thaxter (1895) as 'rare, similar to the oogonia, containing zoospores, 2-ciliate (?) about 10–12μ in diameter' have not been observed. On the other hand, oogonia in which the contents appear to have disintegrated were found. In some cases the disintegrating contents were seen to be leaving the oogonium, only a few large oil globules being left behind. It is interesting to note that Thaxter writes: 'After the escape of the zoospores a large oil globule always remains in the sporangium.' It seems likely that Thaxter either mistook these emerging disintegrating oogonial contents for zoospores, or as Sparrow (1933) suggests, the 'zoospores' were extraneous parasitic organisms that had forced their way into the oogonium to feed on its disintegrating contents—the residual oil-globules, described by Thaxter, being ooplasmic material not available to the parasite.

As with *M. fasciculata*, the smooth endogenous oospores of this species have not been observed to leave the oogonium. Laibach (1927) considered that the endogenous position of the oospores in *M. insignis* (as in *M. fasciculata*) was due to poor environmental conditions. This view seems untenable in the light of experience gained during this investigation, for *M. insignis* has been found growing under the same conditions, and upon the same twig, as *M. macrandra*, *M. polymorpha* and *M. hypogyna*, in which all fertilized and mature oospores were exogenous in position.

Monoblepharis insignis var. **minor** var.nov. (Pl. 10; Text-fig. 7)

Mycelium hyphis rectis, longitudine 1–2 mm., 5–14μ diam. (maior pars 7–9μ).

Oogonia vel singularia vel in ordine disposita, 2–16 numero (maior pars 9), in extremis hyphis: aliquando eadem longitudine 25–54μ (maior pars 36–43μ), 14–36μ diam. (maior pars 21–25μ).

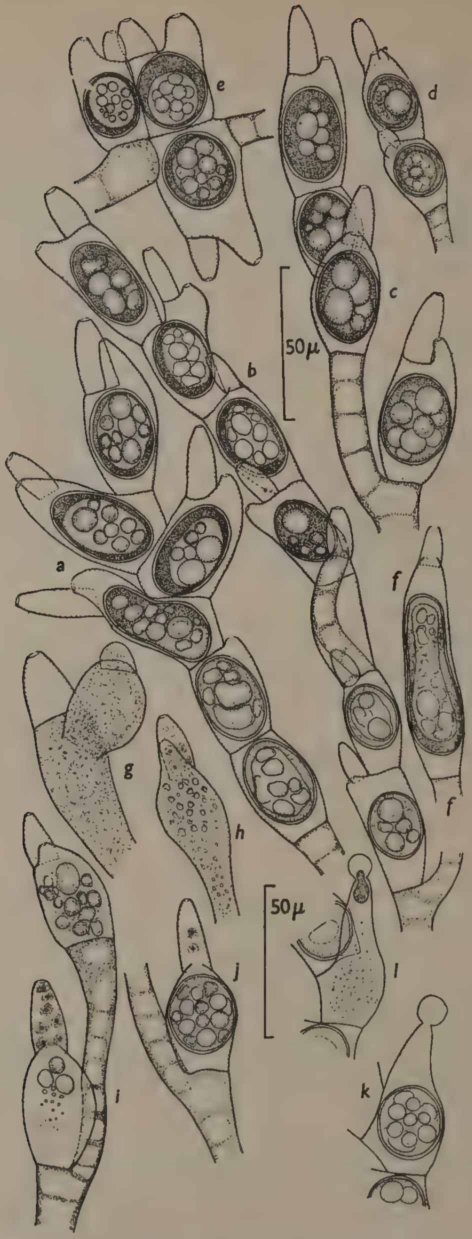
Antheridia varia longitudine 7–29μ (maior pars 14–21μ), diam. 5–14μ (maior pars 7–9μ): plerumque lata in extremo oogonio iuxta 'collum', sed aliquando hypogyna, aditu pert ubulum eminentem sub murum oogonialem crescentem. Antherozooida 5–7 numero, longitudine 3·5–6μ, diam. 3·5μ.

Oosporae longitudine 18–32μ (maior pars 23–25μ), 11–25μ diam. (maior pars 20–21μ), maxime varia magnitudine.

Aliquando adsunt oogonia collis praelongis nullum ferentia antheridium neque ante oosporae maturitatem aperta: quae oosporae interdum constrictae sporam minorem sphaericam, muro levi a se abscidunt et per caveam in extremo oogonio sitam emittunt. Sunt quoque alia oogonia paulo angustiora et parthenogenetica, caveam supradictam non habentia quorum contenta in partes sphaericas, muro levi, dividuntur.

Saprophytes est in ramulis submersis *Quercus roboris*, Dinas Powis, Glamorgan, South Wales, March 1938.

Mycelium well developed consisting of straight, rigid, cylindrical, hyaline, sometimes tinged with brown, vacuolated hyphae, which are simple or branch sympodially. Each hypha bears one oogonium or a superimposed series of oogonia at its tip. Length of hypha 1–2 mm., diameter varying from 5 to 14μ (majority 7–9μ); attached to the substratum by branching rhizoids.



Text-fig. 7. *M. insignis* var. *minor* var.nov. *a* and *b*, a typical arrangement of reproductive organs for this species, a chain of superimposed oogonia, usually bearing epigynous antheridia, borne at the tip of an unbranched hypha. *c*, a sympodially branched hypha bearing its sex organs singly, and in a superimposed series of three. *d*, an unusually small set of reproductive organs. *e*, a set of three intercalary oogonia of unequal size, each with a neck as long as its epigynous antheridium. *f*, an unusually long oogonium. *g*, hypha bearing at its tip two newly formed antheridia. Oogonia are not yet cut off. *h*, antheridium with its antherozooids partially emerged, borne upon an oogonium which is not yet fully formed. *i*, a sympodially branched hypha bearing at its tips single sets of sex organs. The contents of the terminal oogonium are rich in oil globules, but an oosphere is not yet formed. The other oogonium also contains undifferentiated contents, but its epigynous antheridium contains antherozooids. *j*, antheridium still containing antherozooids, and oogonium containing a single, thick-walled oospore. *k*, one oogonium (from a series of typical sex organs), with its neck produced at the tip into a closed spherical chamber. It does not bear an antheridium, and contains a parthenogenetic oospore. *l*, another oogonium (from a series of sex organs) containing undifferentiated contents, a portion of which has collected and is about to fill the spherical chamber at the tip. This oogonium does not bear an anthierdum.

Oogonia arranged singly, or in rows of 2–16 (average 9), formed in basipetal succession, at the tips of the hyphae. The long chains of superimposed oogonia are very characteristic of this variety (Text-fig. 7*a*, *b*). Occasionally intercalary oogonia are formed. Oogonia are usually cylindrical in shape, but occasionally irregular, opening when the oosphere is formed by a tube or 'neck' which may be curved or straight. The neck is very variable, long- and short-necked forms being found in the same series of oogonia. The oogonia vary in length from 25 to 54μ (majority 36–43μ), diameter 14–36μ (majority 21–25μ).

Antheridia normally, but not always, present, varying from subconical to cylindrical in shape, tapering at the tip, narrow, usually straight, but occasionally slightly curved; length 7–29μ (majority 14–21μ); diameter 5–14μ (majority 7–9μ). The majority of antheridia are longer than the neck of the oogonium, although some may be equal in length or shorter than it (Text-fig. 7); as a rule borne upon the terminal wall of the oogonium (epigynous), to one side of the neck, but occasionally hypogynous and intercalary in position, opening by an exserted tube formed just beneath the oogonial cross-wall. A single antheridium usually contains 5–7 amoeboid and uniflagellate *antherozooids* which measure 3·5–7μ by 3·5μ. Protandry is well marked.

Each oogonium contains a single oospore which matures and germinates *in situ* (endogenous). *Oospores* golden brown, smooth, and very variable in shape, spherical, cylindrical, irregular, and widely ellipsoid forms being found (ellipsoid form the most typical); length 18–32μ (majority 23–25μ); diameter 11–25μ (majority 20–21μ). Much variation in oospore size is found, even among oospores contained in oogonia borne on the same filament (Text-fig. 7*b*–*d*).

Oogonia in which the contents disintegrate and completely leave the oogonium, the larger oil globules leaving last, are commonly found (Text-fig. 7*i*). This disintegration seems to occur in oogonia where the oosphere has not been fertilized. Occasionally, oogonia with elongated necks, which terminate in a spherical chamber approximately 10μ in diameter, are to be found among the normal oogonia of a linear series (Text-fig. 7*k*, *l*). These oogonia do not bear an antheridium and do not open until after the single oospore which they contain has matured, which suggests that it is parthenogenetic in its development. Oospores formed in this way have been seen to cut off by constriction, a smaller, spherical, smooth-walled spore, which passes into the chamber at the tip of the oogonium, to be set free.

Cylindrical zoosporangia, as found for the exogenous species of *Monoblepharis*, have not been observed.

On submerged twigs of *Quercus robur* L. and *Fraxinus excelsior* L., St Mellons and Dinas Powis, South Wales.

This variety resembles *M. insignis* var. *insignis* in every way except for its much smaller size and the presence of parthenogenetic oogonia of the type described here for *M. fasciculata*. Material of *M. insignis* var. *minor*, producing large quantities of sexual reproductive organs, was extremely abundant in these collections. It has been found growing under the same conditions, and upon the same twig, as the larger var. *insignis*. The limits imposed on this variety were based on 500 measurements of oospores taken from samples of material collected at different times of the year, from different collecting stations, and on different substrates.

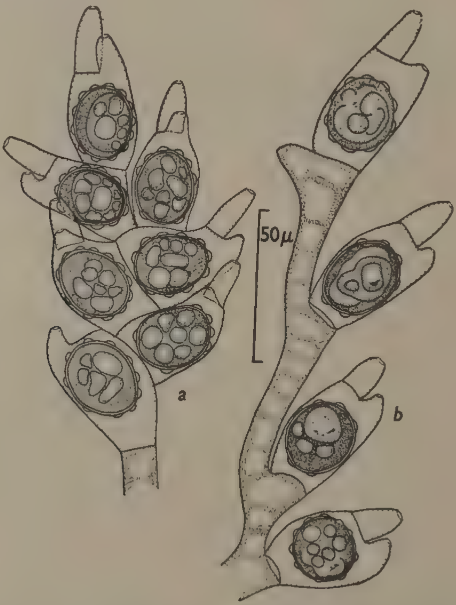
By comparison of data it was seen that environmental changes did not cause differences in the frequency of occurrence of oospore sizes, or in the limits of variation.

Sizes covering the range of *M. insignis* var. *insignis* were seen to separate off from another distinct range of smaller sizes—distinguished as *M. insignis* var. *minor*. The smooth endogenous oospores of the normal oogonia in this variety have not been observed to emerge from the oogonium. It has been found growing upon the same twig as exogenous species of *Monoblepharis*.

Monoblepharis bullata sp.nov. (Pl. 11, figs. 1, 2; Text-figs. 8, 10, 9, 11)

Mycelium hyphis rectis, rigidis, luteis, cylindricis, raro ramosis, longitudine, 1·5–2·5 mm., 5–23μ diam. (maior pars 9μ).

Oogonia aut singularia, in ordine disposita, 2–12 numero, aut in fascibus, in extremis hyphis: paene cylindrica, aditu per tubulum vel rectum vel curvatum, extremo eminente ad antheridium; aliquando informia, longitudine varia 25–53μ (maior pars 34–43μ), 16–42μ diam. (maior pars 21μ).



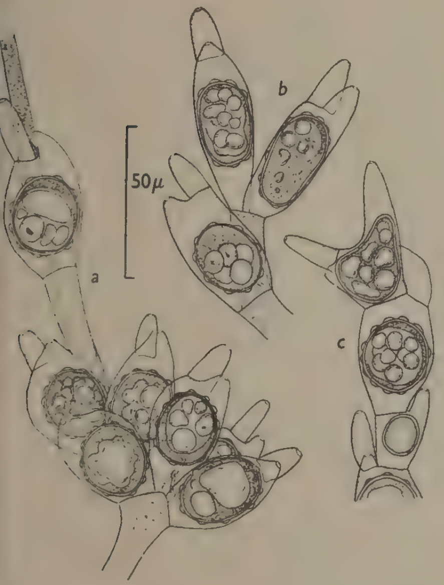
Text-fig. 8. *M. bullata* sp.nov. *a*, a hyphal tip showing a very common arrangement of reproductive organs, i.e. a series of superimposed oogonia. Each oogonium has one of its lateral walls attached to the base of the previously formed oogonium above. Each oogonium contains a single bullated endogenous oospore, and bears an empty epigynous antheridium. *b*, another common arrangement of the reproductive organs, oogonia and their epigynous antheridia, in single sets at the tips of a sympodially branched hypha.

Antheridia forma varia, vel subconica vel cylindrica, recta aut paulo incurvata, longitudine varia etiam in eodem globo, 10·5–25μ (maior pars 14–21μ), 6–14μ diam. (maior pars 7–9μ, contractione ad 5–7μ): plerumque adsunt in extremo oogonio iuxta collum.

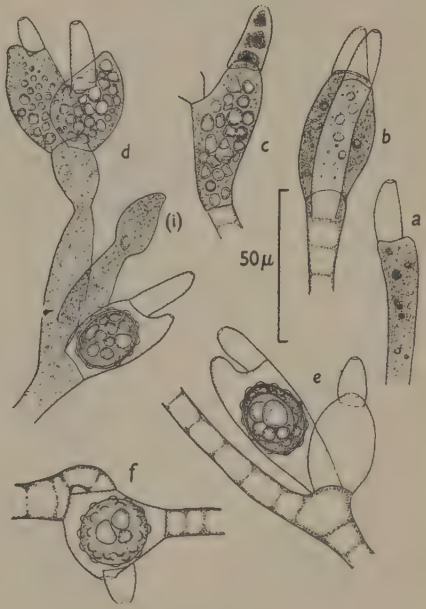
Antherozooida 5–6 numero, 4–6μ longitudine, 4–5μ diam.

Oosporae in oogonio maturescentes, et in situ germinantes, luteae et verrucis parvis (1μ altitudine), subflavis et pellucidis, sphaericis aut ellipsoideis, aliquando informibus: longitudine 19–23μ (maior pars 21–25μ), 12–27μ diam. (maior pars 18–21μ).

Ex oogoniis ut videtur, non impregnatis, omnia quae insunt nonnumquam tota dissolvuntur.



Text-fig. 9.



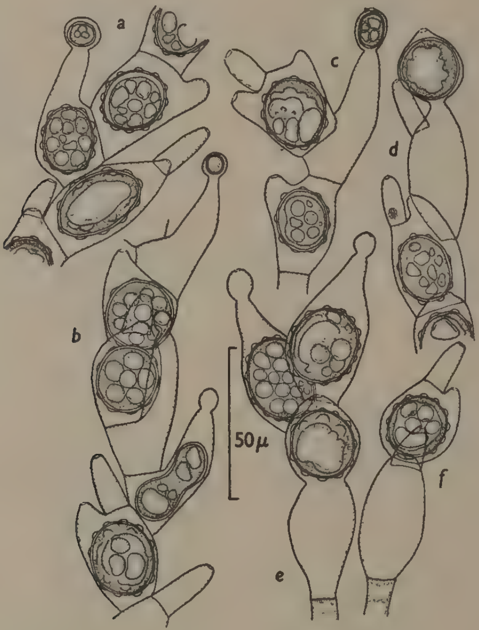
Text-fig. 10.

Text-fig. 9. *M. bullata* sp.nov. *a*, a hypha bearing at its tip six sets of sexual reproductive organs arranged in a fascicle (a common arrangement for this species), while a branch of this hypha bears a single set of reproductive organs. *b*, a group of reproductive organs, with the terminal oogonia and oospores longer than the average. *c*, a portion of an abnormal series of sexual reproductive organs borne at a hyphal tip. The terminal oogonium has two necks, one large and one small, instead of the usual one, and it contains an irregular oospore, without bullations. The second oogonium bears an epigynous antheridium which is very much curved. This oogonium contains an oospore which is normal in size and shape, and bullated. The third oogonium is unusually small, does not bear an antheridium, and contains a very small, smooth-walled oospore. The rest of the oogonia and antheridia (not figured) of the series were normal, all bearing epigynous antheridia and containing single bullated oospores.

Text-fig. 10. *M. bullata* sp.nov. *a*, a hypha which has formed an antheridium at its tip. Beneath its cross-wall the hypha is swelling out, preliminary to the formation of an oogonium. *b*. a hypha with an oogonium and antheridium at its tip. Beneath the oogonial cross-wall, the hypha gives rise to a branch which has cut off an antheridium from its tip. *c*, a young oogonium with contents rich in oil globules, bearing an epigynous antheridium containing three antherozooids. *d*, a hypha bearing two fascicles of reproductive organs. The terminal fascicle consists of two young oogonia in which the contents have not yet formed an oosphere. Each bears an empty epigynous antheridium. The other fascicle consists of a young oogonium (i), not yet fully formed, which will have its neck produced into a spherical chamber as shown in Text-fig. 11. This oogonium does not bear an antheridium. The other oogonium of this fascicle is normal in shape, bears an empty antheridium, and contains a mature bullated oospore. *e*, a sympodially branched hypha bearing at its tip two sets of sexual reproductive organs. One oogonium is unusually long, but contains an average-size, bullated, oospore. The other oogonium has lost its contents. *f*, an intercalary oogonium, containing a mature bullated oospore, and bearing an epigynous antheridium.

Saepe adsunt oogonia collis praelongis nullum ferentia antheridium neque ante oosporae maturitatem aperta: quae oosporae interdum constrictae sporam minorem, sphaericam, muro levi, a se abscidunt et per caveam in extremo oogonio sitam emittunt. Sunt quoque alia oogonia paulo angustiora et parthenogenetica caveam supra dictam non habentia, quorum contenta in partes sphaericas, muro levi, dividuntur.

Saprophytes est in ramulis submersis *Quercus roboris* L., Dinas Powis, Glamorgan, South Wales, March 1938.



Text-fig. 11. *M. bullata* sp.nov. *a*, portion of a series of sexual reproductive organs. All the oogonia are normal except one, which is shown in the figure to have its long neck continued to form a spherical chamber at its tip, which has not yet opened. This contains a small smooth-walled spore formed by constriction from the immature parthenogenetic oospore. The remainder of the oospore has since acquired a bullated exospore. This oogonium does not bear an epigynous antheridium. *b*, another portion of a series of sexual reproductive organs. In this, three normal and three abnormal oogonia are shown. None of the latter bears an antheridium. The terminal oogonium of the figure contains a small spore in the spherical chamber at its tip, while the rest of the oogonium is empty. The fourth oogonium of the series is irregular and is completely empty. The one immediately beneath this latter is closed and contains an immature, smooth-walled, parthenogenetic oospore, which is elongated and seems about to divide by constriction. *c*, a series of three unusual oogonia. The terminal one is irregular in shape, but contains a symmetrical bullated oospore, and bears an epigynous antheridium. The oogonium beneath is long and narrow, bearing a spherical chamber at its tip. This latter contains a small, smooth-walled spore; the rest of the oogonium is empty. The basal oogonium of the series is of the usual shape, but is unusually small in size. *d*, portion of a series of reproductive organs, showing at its tip an elongated oogonium which has an unusually large spherical chamber at its tip, filled by a smooth-walled spore. Unlike other abnormal oogonia, it bears an epigynous antheridium. *e*, a series of three abnormal oogonia, none of which bears an epigynous antheridium. Each of the two terminal oogonia contains a mature bullated oospore in the main body of the oogonium, the small spherical chambers at their tips being empty. The basal oogonium of the series has an unusually large terminal chamber containing a bullated spore, corresponding in size with the majority of oospores. *f*, two oogonia, each bearing an epigynous antheridium. The terminal oogonium is smaller than is normal, and contains a mature oospore; the other oogonium has lost its contents.

Mycelium of straight, rigid, hyaline or pale brown, cylindrical, rarely branched hyphae, 1·5–2·5 mm. long; attached to the substratum by branching rhizoids. Diameter 5 μ (in narrowest parts) to 23 μ (at the tips which are expanded to bear fascicles of sexual reproductive organs); average for intermediate portions 9 μ.

Oogonia arranged singly (Text-fig. 8*b*), in superimposed series of 2–12 (Text-fig. 8*a*) or in fascicles of 2–8 (Text-fig. 9*a*), borne at the tips of the hyphae or their sympodial branches. Sometimes combinations of these three arrangements are to be found on one hypha; occasionally intercalary (Text-fig. 10*f*). Oogonia usually cylindrical (Text-fig. 8*a*, *b*), opening when the oosphere is formed at the tip of the prominent neck, which may be straight (Text-fig. 9*b*) or curved, with its tip pointing towards the antheridium (Text-fig. 8*a*). Oogonia occasionally exhibit irregularities of form and size (Text-fig. 9*c*). Length 25–53 μ (majority 34–43 μ), diameter 16–42 μ (majority 21 μ).

Antheridia tapering at the tips, and varying from subconical to cylindrical (Text-fig. 8). Narrow, straight or slightly curved, with the tip bent inwards towards the neck of the oogonium. Much variation in length even among those in the same group. 10·5–25 μ long (majority 14–21 μ) by 6–14 μ diameter (majority 7–9 μ tapering to 5–7 μ). Not always present, but usually so (Text-fig. 11*a*, *b*). Borne upon the terminal wall of the oogonium (epigynous), to one side of the neck. Occasionally hypogynous and intercalary. Each usually contains 5–6 amoeboid and uniflagellate *antherozooids* which measure 4–6 μ by 4–5 μ. Protandry is well marked.

Each *oogonium* contains a single oospore which matures and germinates within the oogonium (endogenous). The *oospores* have thick, golden brown walls covered with small, pale yellow, transparent bullations which are 1 μ in height. They vary in shape from spherical to widely ellipsoid (Text-figs. 8, 9), and are occasionally irregular in outline (Text-fig. 9*c*); length 19–32 μ (majority 21–25 μ), diameter 12–27 μ (majority 18–21 μ). Much variation in size is often seen in the same series.

On submerged twigs of *Quercus robur* L. and *Fraxinus excelsior* L., Dinas Powis, Glamorgan, South Wales.

Oogonia in which the contents disintegrate and leave the oogonium are often found, the disintegration seeming to occur in oogonia that have not been fertilized.

Oogonia with elongated necks that terminate in a spherical chamber, varying in length from 8 to 14 μ, and in diameter from 6 to 10 μ, are frequently found among the normal oogonia of a superimposed series (Text-fig. 11). These abnormal oogonia do not usually bear an epigynous antheridium, and never open until after the single oosphere which they contain has formed an exospore, which suggests that development is parthenogenetic. Before bullations are formed on the exospore, i.e. presumably when the oospore is not yet fully mature, a small spherical spore 5–11 μ by 5–9 μ is often seen to be formed by constriction from the oospore. This passes into the chamber at the tip of the oogonium, from which it is set free (Text-fig. 11*a*, *b*). This type of oogonium is also to be found in *M. fasciculata* and *M. insignis* var. *minor*. Occasionally, in *M. bullata* this chamber at the oogonial tip is seen to be very much enlarged, reaching

26–28 μ in length by 22–24 μ in diameter, and containing a spore corresponding in size with the original oospore, 20–23 μ in length by 20 μ in diameter (Text-fig. 11*d*, *e*).

M. bullata also possesses another type of oogonium, a little narrower than the normal, and without a spherical chamber at the tip. The contents divide into separate, spherical, smooth-walled spores which mature before the oogonium has opened. These resemble the parthenogenetic oospores described for *M. polymorpha* and *M. macrandra*.

Long, narrow, cylindrical zoosporangia, as found in the exogenous species of *Monoblepharis*, have not been observed.

M. bullata resembles *M. insignis* var. *minor* and *M. fasciculata* in the following characters: oogonial shape, endogenous oospores, epigynous antheridia, oogonial arrangement, size, and in the possession of parthenogenetic oospores. It differs from these species in the possession of bullated oospores in which it resembles *M. sphaerica*.

M. bullata was found growing abundantly during this investigation with other species of the genus.

The limits imposed on this species were based on 500 measurements of oospores taken from samples of material collected at different times of the year, from different collecting stations and on different substrates.

Monoblepharis sphaerica Cornu, *Bull. Soc. bot. Fr.* **18**, 58–59, 1871, and *Ann. Sci. nat. Bot.* **15**, 1–198, 1872. Non *Monoblepharis sphaerica* Cornu emend. Woronin, *Mém. Acad. Sci. St Pétersb.* **16**, 1–24, 1904. (Pl. 3, figs. 3–4; Text-fig. 12)

On submerged twigs of *Quercus robur* L., Dinas Powis, Glamorgan, South Wales.

It has not been described since its original description by Cornu in 1871. This original description contains few measurements, and was apparently compiled after the examination of a small amount of material. Therefore a full description is included here.

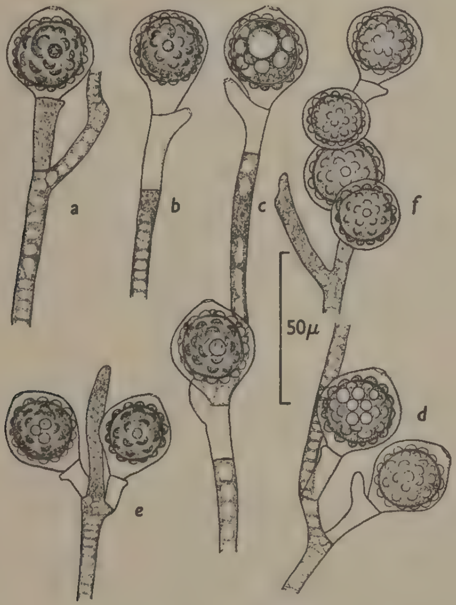
The mycelium is well developed, consisting of cylindrical, somewhat rigid, rather frequently branched hyphae, 1–2 mm. long by 4–6 μ in diameter.

Oogonia are subspherical to spherical, usually arranged singly at the tips of the hyphae and their branches (Text-fig. 12*a*–*c*); occasionally borne in fascicles at the tips of the branches (Text-fig. 12*e*), and less frequently in a series alternating with hypogynous antheridia (Text-fig. 12*f*). They vary in length from 21 to 31 μ (majority 23 μ), diameter 19–27 μ (majority 21 μ).

Antheridia are narrowly cylindrical, hypogynous, always present, usually opening by a slightly exserted tube approximately 2 μ long, formed just below the oogonial cross-wall (Text-fig. 12*a*, *e*). Occasionally this tube is curved and reaches 4–10 μ in length (Text-fig. 12*c*). Antheridia 10–23 μ long (majority 15 μ) by 4–6 μ in diameter. *Antherozooids*, amoeboid, uniciliate, 3·5 × 2 μ, 4–7 in a single antheridium; usually protandrous.

Each oogonium contains a single spherical oospore, not completely filling the oogonium. The *oospores* are endogenous and germinate *in situ*. They are thick-walled, golden brown in colour, and covered when mature with closely arranged, colourless to pale yellow bullations, 1–2 μ in height. Diameter of oospore varies from 12·5 to 23 μ (majority 17–19 μ).

On rare occasions oospores have been seen to constrict, forming smaller spores, 8–11 μ in diameter, which are set free, as seen in the parthenogenetic oospores of *M. bullata*, *M. polymorpha* and *M. macrandra*. Oogonial contents which disintegrate and leave the oogonium have also been observed, this seeming to occur where fertilization has not taken place.



Text-fig. 12. *M. sphaerica*. *a*, sympodially branched hypha bearing one antheridium and one oogonium. The antheridium is hypogynous in position, and its contents are not differentiated to form antherozooids. The oogonium contains one endogenous bullated oospore. *b*, hypha bearing at its tip an empty hypogynous antheridium, the exit tube of which is only slightly exserted, and an oogonium containing one mature, endogenous oospore. *c* and *d*, a typical arrangement of the sexual reproductive organs, one oogonium and its hypogynous antheridium being borne at the tip of each sympodially formed branch. *e*, two oogonia and their hypogynous antheridia being borne side by side at the hyphal tip, another fairly common arrangement. *f*, an uncommon arrangement of the reproductive organs.

Oospore sizes were based on 500 measurements made on material collected at the same and different times of the year, on different substrates and from different collecting stations. Sets of measurements were compared, and it was shown that environmental changes did not affect the frequency of occurrence of oospore sizes, or the limits of variation.

The fungus named *M. sphaerica* here agrees with the original description at every point, and it is obvious on examination of Cornu's figures (1872) that it is the same.

In 1904, Woronin emended Cornu's description of *M. sphaerica* to include a form he described from Finland. This fungus resembled *M. sphaerica* Cornu in the possession of bullated oospores of a similar size, and in the possession of hypogynous antheridia, but it differed from Cornu's species in the following characteristics:

(1) When mature the fully bullated oospores were exogenous and attached to the oogonial mouth, as in *M. macrandra* and *M. polymorpha.* When endogenous oospores occurred, they were bullated only upon the upper and lower surfaces, the lateral surfaces being smooth and closely pressed against the oogonial wall (Text-fig. 13*h*), as is often the case in immature oospores of exogenous species during the process of emergence (Text-figs. 13*d*, 14*e*).

(2) The oogonia are pyriform, and usually narrowly so, never spherical.

(3) The hyphae are rarely branched.

Because Woronin (1904) held the view that under favourable environmental conditions oospores of all species of *Monoblepharis* would be exogenous, he regarded Cornu's species as being the same as the form he described. During the present investigation abundant material of *M. sphaerica* Cornu was collected at the same time, and grown under the same conditions, as the exogenous species, including the form described by Woronin. Therefore, a new species, *M. hypogyna*, has been erected to include the exogenous species described as *M. sphaerica* by Woronin, and Cornu's description for *M. sphaerica* is adhered to.

Monoblepharis hypogyna sp.nov. (Text-fig. 13.) *Monoblepharis sphaerica* Cornu emend. Woronin, *Mém. Acad. Sci. St Pétersb.* **16**, 1–24, 1904 pro parte

Mycelium amplum, hyphis cylindricis, rigidis, plerumque raro ramosis, longitudine 1–2 mm., 7·5μ diam. in partibus crassioribus, angustioribus in partibus extremis, 2μ diam. (maior pars 3–5μ diam.).

Oogonia pyriforma et angusta; aut singularia terminaliaque aut ordine aeque disposita antheridiis alternis; aliquando intercalaria; longitudine 21–45μ (maior pars 25–34μ), 8–19μ diam. (maior pars 13μ).

Antheridia cylindrica et angusta, hypogyna, aditu per tubulum paulo eminentem et sub murum oogonialem crescentem, longitudine 9–27μ, 4–9μ diam. Antherozooida 4–7 numero, circa 3·5μ longitudine, 1·5–2μ diam.

Oosporae plerumque extra oogonium maturescentes, sphaericae, muro crasso, luteae, 13–23μ diam. (maior pars 17μ), verrucis pallidis, 2μ altis; aliquando inclusae in oogonio: germinatio per hypham.

Aliquando sunt corpora lutea, muro levi, inclusa maturescentia in oogonio longitudine 28–31μ, 15μ diam.; quae 'chlamydosporae' appellata sunt.

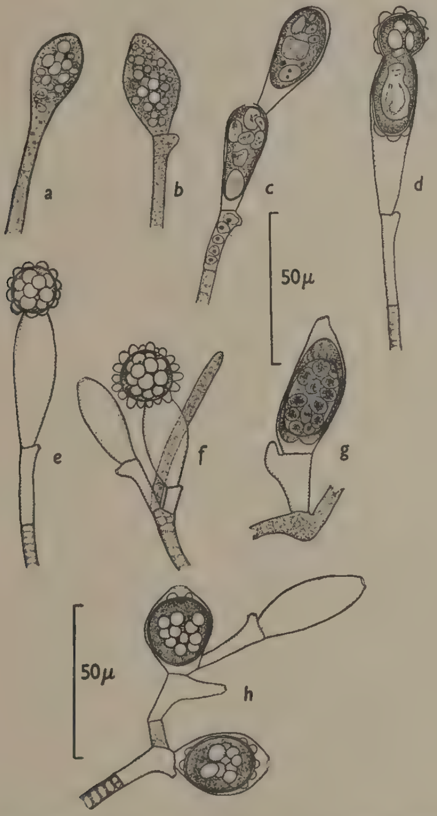
Zoosporangia cylindrica et angusta, aut terminalia singulariaque (longitudine 72–104μ: 5–7μ diam.), aut ordine disposita aequis intervallis (longitudine 21–38μ; 5–7μ diam.). Zoospora 4–9μ longitudine; 3–5μ diam.

Saprophytes est in ramulis submersis *Quercus roboris*, St Mellons, Glamorgan, South Wales, March 1938.

Mycelium well developed, consisting of cylindrical, somewhat rigid, usually sparingly branched hyphae 1–2 mm. long, and varying in diameter from 7·5μ in the stouter basal portions to 2μ at the tip (average 3–5μ).

Oogonia narrowly pyriform, occurring singly and terminally or occasionally in a series alternating with antheridia, or in a fascicle, at a hyphal tip; rarely intercalary. Length 21–45μ (majority 25–34μ); diameter 8–19μ (majority 13μ) (Text-fig. 13*a*–*f*).

Antheridia narrowly cylindrical, hypogynous, opening by a slightly exserted tube just beneath the oogonial cross-wall (Text-fig. 13*c–f*). Length, 9–27μ; diameter 4–9μ. An antheridium is nearly always present at the base of each oogonium, except in the case of some series of oogonia



Text-fig. 13. *M. hypogyna* sp.nov. *a*, hypha swollen at its tip with dense protoplasm rich in oil globules, preliminary to the formation of an oogonium. *b*, a very young oogonium recently cut off from the rest of the hypha by a cross-wall. The portion immediately beneath it is about to form an antheridium. *c*, hypha bearing two superimposed oogonia, each containing an oosphere rich in oil globules. The hypogynous antheridium contains five antherozooids. *d*, hyphal tip bearing an empty hypogynous antheridium, and an oogonium from which the partly bullated oospore is beginning to emerge. *e*, an oogonium bearing at its mouth the mature exogenous bullated oospore. *f*, a sympodially branching hyphal tip bearing two sets of reproductive organs. One oogonium has lost the mature exogenous oospore from its mouth, while the other still has its exogenous oospore attached. *g*, a large oogonium which contains an abnormal oospore which formed its thick smooth wall before the oogonium opened. *h*, hypha bearing three sets of reproductive organs at its tip. The antheridia are empty, and two of the oogonia contain endogenous oospores which are not fully bullated.

and antheridia, where the hypha will occasionally give rise to two oogonia without the alternation of an antheridium (Text-fig. 13*c*). A single antheridium usually contains 4–7 amoeboid uniflagellate *antherozooids* approximately 3·5μ by 1·5–2μ.

Each oogonium produces a single spherical oospore, 13–23μ in diameter (majority 17μ), and is nearly always exogenous when mature. These *oospores* are spherical in shape, thick-walled, golden brown in colour, and covered at maturity with light yellow, transparent bullations, 2μ in height (Text-fig. 13*e*, *f*). When the oospore reaches the outside of the oogonium it remains attached to the oogonial mouth for some time before it falls away. When endogenous oospores are found, the oospore is seen to fill the diameter of the oogonium, and bullations are to be found only upon those surfaces of the oospore which are not adpressed to the walls of the oogonium (Text-fig. 13*h*). Oospores produce a vegetative hypha on germination.

Occasionally oogonia containing light brown, smooth, oval, thick-walled, endogenous spores, 28–31μ in length by 15μ in diameter are found. They are seen to mature, i.e. to form thick walls, before the oogonium opens, and in all probability they are parthenogenetic oospores, similar to those found in *M. polymorpha*, *M. macrandra* and *M. bullata*. They have been called 'resting bodies' and 'chlamydospores'.

Zoosporangia are narrowly cylindrical, 72–104μ in length by 5–7μ in diameter when borne singly in a terminal position, or 21–38μ in length by 5μ in diameter when borne in a row. Occasionally borne in an intercalary position. *Zoospores*, amoeboid, uniflagellate, 4–9μ by 3–5μ, arranged in a single or double row, inside the sporangium.

On submerged twigs of *Quercus robur* L., St Mellons, South Wales. Subsequently collected from ponds at Dinas Powis and Castleton, South Wales, and Styal, Cheshire.

The first record of this species was made by Woronin in 1904 under the name *M. sphaerica* Cornu emend. Woronin. In this description the characters of *M. sphaerica* were incorporated with those of his new form. For example, the oogonia were described as 'pyriform to subspherical' to include the pyriform oogonia of this exogenous form, and the subspherical to spherical oogonia of Cornu's endogenous species. The only measurements given by Woronin were 'oospores, average diameter 22μ', although the fungus was extensively figured. On examination of these figures it was obvious that Woronin was describing the species designated *M. hypogyna* here.

Following Woronin's description, *M. hypogyna*, described as *M. sphaerica* Cornu emend. Woronin, has been recorded from Germany, Latvia, England, U.S.A. and Denmark (see Table 1).

It was decided to erect a new species for this exogenous form because:

(1) When mature, the oospores of *M. hypogyna* are usually exogenous, fully bullated, and attached to the oogonial mouth, as in *M. polymorpha* and *M. macrandra* (Text-fig. 13*e*). When occasional endogenous oospores are found they are only partially bullated (Text-fig. 13*h*), the lateral surfaces remaining smooth, and closely pressed against the oogonial wall, as in immature oospores during their emergence from the oogonium (Text-fig. 13*d*). In *M. sphaerica* Cornu, on the other hand, the oospores are seen to be constantly endogenous, spherical, fully bullated, and not completely filling the oogonium (Text-fig. 12), thus giving the fungus quite a different appearance from *M. hypogyna*.

(2) The oogonia of *M. hypogyna* are narrowly pyriform (Text-fig. 13*e*), and never spherical, while in *M. sphaerica* Cornu they are always subspherical to spherical.

(3) The hyphae of *M. hypogyna* are rarely branched, while those of *M. sphaerica* are frequently branched.

The first and most important point of difference was disregarded by Woronin because of his view that under favourable environmental conditions the oospores of all species of *Monoblepharis* would be exogenous. During this investigation *M. hypogyna* has been found growing with *M. sphaerica* in the same collections and even upon the same twig. Also the two species have been grown in the laboratory under identical conditions. In each case all the oospores of *M. sphaerica* remained endogenous, and all the oospores of *M. hypogyna* became exogenous.

Monoblepharis macrandra (Lagerheim) Woronin, *Mém. Acad. Sci. St Pétersb.* **16**, 1–24, 1904.

Monoblepharis macrandra (Lagerheim) Woronin var. **macrandra** (Pl. 13; Text-figs. 1, 2, 14 and 15)

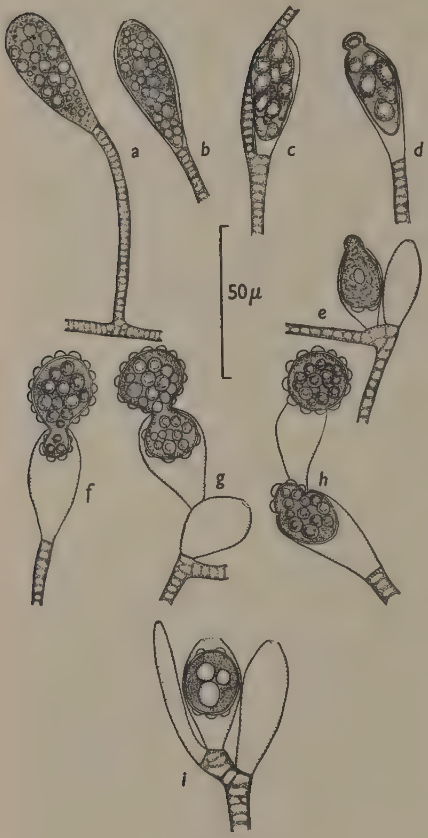
Syn. *Monoblepharis polymorpha* var. *macrandra* Lagerheim, *Bih. svensk. VetenskAkad. Handl.* **25**, 1–42, 1899.

Monoblepharis polymorpha Cornu, *Ann. Sci. nat. Bot.* **15**, 1–198, 1872, pro parte.

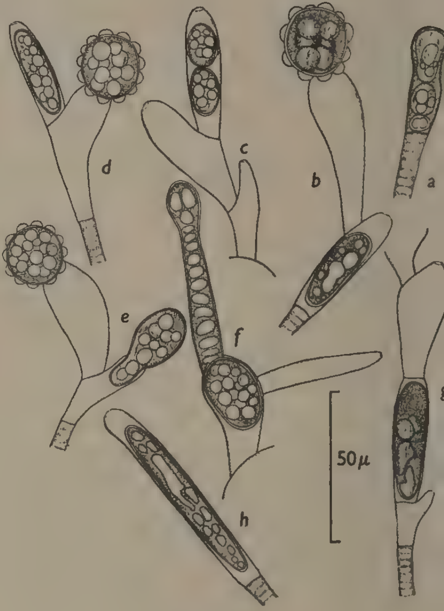
This was the most abundantly occurring species of the genus in this investigation, having been found at every collecting station at Dinas Powis, St Mellons and Castleton, South Wales on twigs of *Quercus robur* and *Fraxinus excelsior*. It has also been collected by the author from ponds at Keele, North Staffordshire on twigs of *Quercus robur*, and from Doddington Mere, Cheshire on twigs of *Ulmus* sp. This, together with *M. polymorpha*, is also the species most frequently recorded by other authors (see Table 1).

The material found agrees with previous descriptions of the species in every respect except one, namely the lengths recorded for the zoosporangia. Sparrow (1933), who is the only author who has recorded sporangial measurements, gives them as varying from 45 to 103μ. Lengths recorded here vary from 107 to 346μ (Text-figs. 1, 2). This discrepancy may be accounted for by the inclusion of long antheridia as sporangia by Sparrow, for antheridia varying from 12·5 to 59μ in length have been recorded from these collections. Antheridia of 45–59μ, which are often found at the tip of superimposed linear series of antheridia, resemble zoosporangia in every way, except in the size of the antherozooids, which are smaller than zoospores, and in their different behaviour on emergence. In this connexion it is worthy of note that the sporangial lengths recorded by Sparrow for *M. polymorpha*, a species of much the same size as *M. macrandra*, but where antheridia cannot be confused with sporangia because of their epigynous position, vary from 130 to 234μ.

It has been noted that in this species light yellow, transparent bullations 1–2μ in height (majority 2μ) begin to cover the oospore wall immediately after fertilization, and that, by the time the oospore emerges, the



Text-fig. 14.



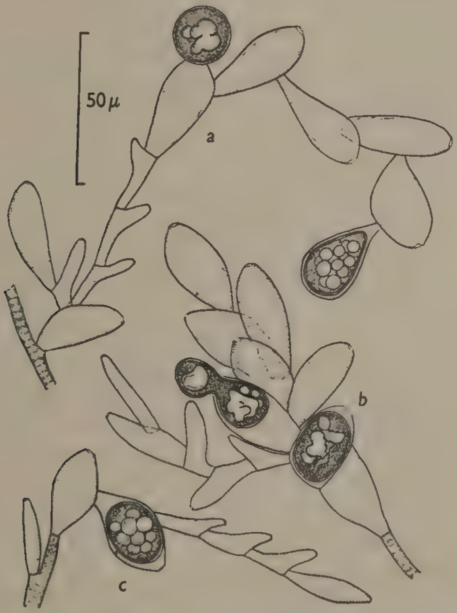
Text-fig. 15.

Text-fig. 14. *M. macrandra* var. *macrandra*. *a*, young oogonium filled with protoplasm rich in oil globules; *b*, oosphere forming and oogonium opening at the tip for entry of antherozooid. *c*, oogonium containing a recently fertilized oosphere. *d*, newly formed oospore beginning to evacuate oogonium. *e*, immature oospore with bullations beginning to form. *f* and *g*, later stages in the evacuation of the oospore from its oogonium. Oospore now completely bullated. *h*, terminal oogonium bearing a mature oospore at its mouth; *i*, hyphal tip which is branching sympodially. At first this hypha bore a single terminal oogonium which is now empty, the oospore having fallen away from its mouth. The branch then formed at its tip one oogonium and one antheridium. The antheridium is now empty, and the oogonium contains an endogenous oospore which is incompletely bullated.

Text-fig. 15. Abnormal oogonia. *M. macrandra* var. *macrandra*. *a*, a terminal closed oogonium, the contents of which seem about to split into two. *b*, a cylindrical oogonium containing a smooth-walled oval oospore with a normal terminal oogonium. *M. polymorpha*. *c*, a cylindrical oogonium containing two smooth-walled oospores. *d*, one normal and one abnormal oogonium. The latter is small and cylindrical, similar in size, shape and position to the antheridium, and contains a smooth-walled elongated oospore. *e*, two oogonia, one normal in shape and size, the other narrow and cylindrical. *f*, a normal oogonium bearing an abnormally long antheridium, and an oogonium of unusual shape containing a parthenogenetic oospore. *g*, an intercalary, cylindrical, closed oogonium, which contains a long, oval, smooth-walled parthenogenetic oospore. *h*, a long, terminal, cylindrical oogonium, containing one elongated oval, smooth-walled oospore.

bullation is complete (Text-fig. 14). On rare occasions endogenous oospores, which like the immature oospores are only partially bullated, with their smooth lateral surfaces closely adpressed to the oogonial wall, are to be found (Text-fig. 14*i*). This is a similar type of endogenous oospore to that noted for *M. hypogyna* and has not previously been described for this species.

Another feature not previously recorded for this species is the occasional occurrence of oogonia containing smooth-walled oospores, which appear to mature before the oogonium has opened. Occasionally these are seen to divide before emergence (Text-fig. 15). They seem to be parthenogenetic in development and resemble the parthenogenetic oospores of *M. hypogyna*, *M. polymorpha* and *M. bullata*. Contents of unfertilized oogonia have also been seen to disintegrate and leave the oogonium.



Text-fig. 16. *M. macrandra* var. *laevis*. *a*, a series of sexual reproductive organs. The terminal oogonium is closed and contains a parthenogenetic oospore, another oogonium has its mature oospore still attached to the oogonial mouth, the remaining oogonia have lost their oospores. The empty antheridia are hypogynous in position and arranged in a superimposed series. *b*, hyphal tip bearing two series of sexual reproductive organs. Six of the oogonia are empty and the oospores have fallen away from the oogonial mouths. One oogonium is in the process of evacuating its oospore. *c*, hypha bearing at its tip a series of two oogonia and four antheridia.

Monoblepharis macrandra var. **laevis** Sparrow, *Ann. Bot., Lond.*, **47**, 517–540, 1933. (Pl. 12, fig. 4; Text-fig. 16)

This variety was found growing abundantly on submerged twigs of *Quercus robur* and *Fraxinus excelsior* at Dinas Powis, St Mellons and Castleton, South Wales, at Keele, North Staffordshire and at Styal, Cheshire.

The material examined agreed with Sparrow's description, but also occasionally bore oogonia containing smooth-walled oospores, which mature before the oogonium has opened (Text-fig. 16).

Monoblepharis polymorpha Cornu, *Bull. Soc. bot. Fr.* **18**, 58–59, 1871; *Ann. Sci. nat. Bot.* **15**, 1–198, 1872. (Pl. 12, fig. 1; Text-figs. 15, 17)

Syn. *Monoblepharis brachyandra* Lagerheim, *Bih. svensk. VetenskAkad. Handl.* **25**, 1–42, 1899.

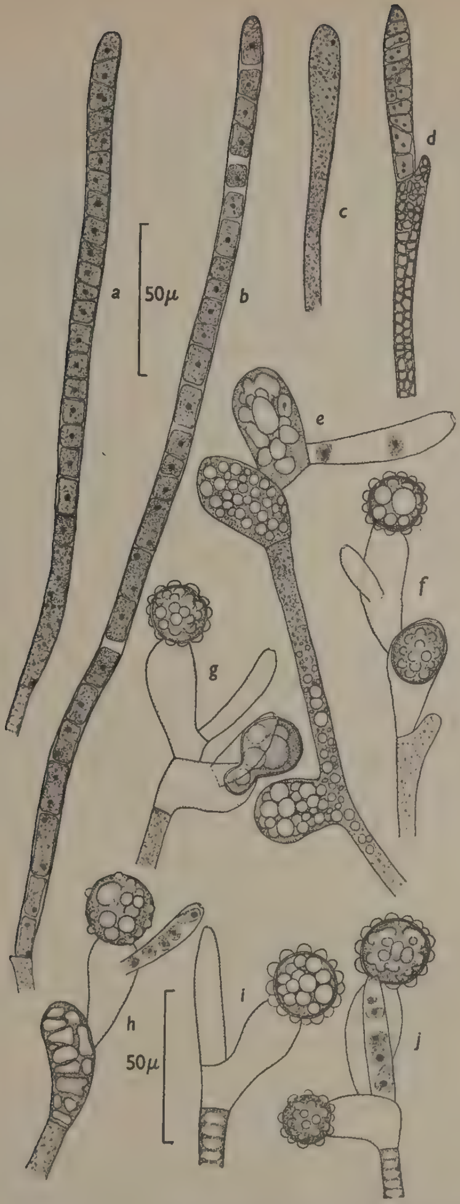
Monoblepharis brachyandra var. *longicollis* Lagerheim loc. cit. p. 38, 1899.

This species was first recorded for Great Britain by the author (Thomas, 1939), although it is the most frequently recorded species from other countries (see Table 1). It was found growing as a saprophyte on *Quercus robur* L. and *Fraxinus excelsior* L. at Dinas Powis, St Mellons and Castleton, South Wales.

M. polymorpha, which occurred in abundance throughout this investigation, was one of the first discovered species of the genus, although its original description by Cornu in 1871 was sufficiently inclusive to embrace *M. macrandra*. *M. polymorpha* differs from the other exogenous species in the possession of epigynous antheridia, in the delay of nuclear fusion until the zygote has reached the oogonial orifice, and in the later maturation of the oospore, this latter point not being noted by other authors. In *M. polymorpha* the zygote wall remains thin and smooth during emergence from the oogonium, and only when it has reached the oogonial orifice is the formation of the thickened bullated exospore commenced (Text-fig. 17*f*–*i*). In *M. macrandra* (Text-fig. 14*e*–*g*) and *M. hypogyna* (Text-fig. 13*d*) a bullated exospore begins to form immediately after fertilization and nuclear fusion, which take place within the oogonium. The fact that Lagerheim described the bullations in *M. polymorpha* as irregular suggests that he was describing immature oospores in which the bullations were not fully formed (Text-fig. 17*h*), as in all the mature oospores observed the bullations are regularly arranged (Text-fig. 17*i*). Another feature, not previously described for this species, is the occasional occurrence of unusually shaped oogonia (Text-fig. 15*a*, *b* and *e*–*h*) containing smooth-walled oospores 25–46μ long by 6–9μ wide, which mature before the oogonium has opened. These are parthenogenetic, and are sometimes seen to divide before emergence into spores 12·5–13·5μ long by 9μ wide (Text-fig. 15*b*). The parthenogenetic oospores noted and figured by Lagerheim for this species resemble the parthenospores of *M. hypogyna* more closely than the type of parthenogenetic oospore observed here.

DISCUSSION

The species of *Monoblepharis* studied during this investigation show interesting differences in the behaviour and maturation of the oospore. In the endogenous species where fertilization and complete nuclear fusion takes place within the oogonium, the zygote is motionless. On the other hand, in the exogenous species fertilization takes place within the oogonium, but complete nuclear fusion and exospore formation are delayed until



Text-fig. 17. *M. polymorpha.* *a* and *b*, a young sporangium, the contents of which have recently divided. (*c–e. Stages in the development of antheridia and oogonia.*) *c*, hypha swollen at its tip, containing dense protoplasm, many nuclei and oil droplets, prior to the formation of sexual reproductive organs. *d*, an antheridium formed from a hypha as shown in *c*, with contents beginning to divide. Oil droplets have accumulated, forming globules, in the portion of the hypha beneath the antheridium, which is swelling to form an oogonium. *e*, hypha bearing at its tip, two young oogonia and one antheridium, which has partly shed its antherozooids; lower down, a young intercalary oogonium, which has not yet become cut off from the rest of the hypha by a cross-wall. (*f–i. Stages in the maturation of the oospore.*) *f*, two oogonia borne at the tip of a hypha. One is empty and has its ripe, evacuated oospore attached to its mouth, while the other contains a recently fertilized oosphere around which a smooth wall is formed. *g*, two oogonia borne at the tip of a hypha. The lower one shows a stage in the evacuation of its smooth-walled oospore. *h*, two oogonia borne at the tip of a hypha. The terminal oogonium shows its evacuated, almost ripe, oospore attached to the oogonial mouth. Once outside the oogonium, the bullations start to form on the oospore wall, but they are not of full size. The oogonium bears an antheridium containing antherozooids. *i*, a single ripe oogonium with its evacuated oospore attached to the oogonial mouth. The bullations of the oospore are fully formed. The oogonium bears an empty antheridium. *j*, two oogonia, each bearing at its mouth its evacuated oospore. These oospores are very unequal in diameter and in the height of their bullations.

the zygote has begun to move towards the oogonial orifice (*M. macrandra* and *M. hypogyna*), or until it has reached the outside of the oogonium, although remaining attached to the oogonial orifice (*M. polymorpha*).

It would seem that in *M. polymorpha* we have the closest link with the related genus *Monoblepharella*. In this genus fertilization takes place within the oogonium, but complete nuclear fusion and exospore formation is delayed for a longer period than in *M. polymorpha* during which the zygote leaves the oogonium and swims by means of the persistent flagellum of the antherozooid (Sparrow, 1940; Shanor, 1942; Springer, 1945). In this respect the genus *Monoblepharella* would seem to occupy an intermediate position between the genera *Monoblepharis* and *Gonapodya*, for in the latter, according to Johns & Benjamin (1954), fertilization may take place either outside the oogonium or within it, and complete nuclear fusion and exospore formation is even longer delayed than in *Monoblepharella*, during which time the zygote swims by means of the persistent flagellum of the antherozooid.

I am much indebted to the late Dr W. R. Ivimey Cook, who suggested this investigation, for his interest and advice. For much helpful advice and criticism in the preparation of this paper I wish to express my thanks to Prof. A. R. Gemmell, Prof. C. W. Wardlaw, and Miss E. M. Blackwell. To Mr I. M. Barton my grateful thanks are due for help in the preparation of the Latin diagnoses. Thanks are due to Mr E. Ashby, Department of Cryptogamic Botany, University of Manchester, for the photomicrographs.

NOTE

The text-figures were drawn with the aid of a camera lucida from unstained living material fixed and temporarily mounted in 4 % formalin, except for Text-figs. 1, 2 and 17*a*, *b*, *d*, *e* and *h*, which were drawn from material fixed in 1 % Osmic acid fumes and stained in a 0·005 % aqueous solution of Crystal Violet using Cotner's method (see text).

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EXPLANATION OF PLATES 9–13

PLATE 9

Fig. 1. *Monoblepharis fasciculata* var. *fasciculata*. Hyphae with sexual reproductive organs borne singly or in fascicles at tips. × 82.

Fig. 2. *M. fasciculata* var. *fasciculata*. Detail of hyphal tips. Most of the oogonia contain single, smooth-walled, endogenous oospores. In the basal oogonium of the topmost fascicle the oosphere has not yet formed. The oogonium on the right contains a thin-walled oosphere. × 330.

PLATE 10

Fig. 1. *M. insignis* var. *minor* var.nov. A typical arrangement of the reproductive organs—chain of superimposed oogonia, mostly bearing epigynous antheridia. × 330.

Fig. 2. *M. insignis* var. *minor*. Hypha bearing a single oogonium with undifferentiated contents, the epigynous antheridium containing antherozooids. × 405.

Fig. 3. *M. insignis* var. *minor*. Another common arrangement of the reproductive organs, oogonia and epigynous antheridia borne singly at the tips of a sympodially branched hypha. In the right-hand corner is an empty oogonium with a spherical chamber at the tip. × 330.

PLATE 11

Fig. 1. *M. bullata* sp.nov. Portion of a series of sexual reproductive organs. All the oogonia are normal except one at the left of the picture. This has the long neck continued to form a spherical chamber at the tip. The chamber contains a smooth-walled spore formed by constriction from the parthenogenetic oospore. × 405.

Fig. 2. *M. bullata.* A series of reproductive organs in which the terminal oogonium bears an antheridium in which there are two remaining antherozooids. The second oogonium contains a young oospore with a thin wall, which is not yet bullated, and bears an exceptionally long antheridium containing antherozooids. The third oogonium of the series shows a mature bullated oospore. × 405.

Fig. 3. *M. sphaerica* Cornu. Hypha bearing at its tip a single oogonium containing one mature endogenous oospore and an empty hypogynous antheridium, the exit tube of which is only slightly exserted. × 330.

Fig. 4. *M. sphaerica.* A typical arrangement of the sexual reproductive organs, an oogonium and hypogynous antheridium being borne at the tip of each sympodially formed branch. × 330.

PLATE 12

Fig. 1. *M. polymorpha* Cornu. Hyphal tip bearing three sympodially arranged oogonia with cylindrical epigynous antheridia and spherical bullated exogenous oospores. × 330.

Fig. 2. *M. polymorpha.* Terminal oogonium showing uneven contour at the place of insertion of the antheridium and bearing an antheridium which is longer than is normal. × 330.

Fig. 3. *M. polymorpha.* The second oogonium of this series of reproductive organs has lost the oospore from the tip and bears a geniculate antheridium. × 330.

Fig. 4. *M. macrandra* var. *laevis* Sparrow. Hyphal tips bearing oogonia which have mature smooth-walled oospores attached to the oogonial mouth in most cases. Oospores have fallen away from the oogonium in two cases. × 330.

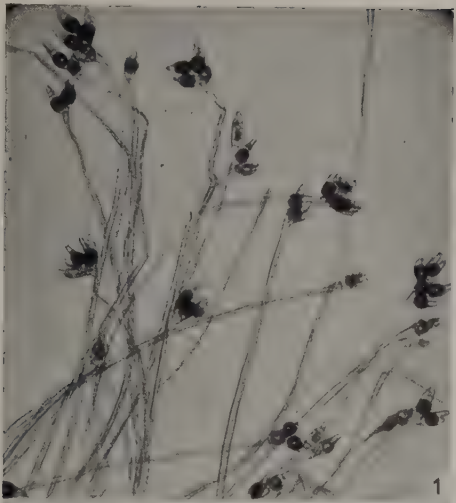
PLATE 13

Fig. 1. *M. macrandra* var. *macrandra.* Young hyphal tip which is swollen prior to the formation of an oogonium, and two young oogonia filled with protoplasm rich in oil globules. (In one oogonium the contents are beginning to round off.) Also oogonium showing a stage in the evacuation of the oospore. × 330

Fig. 2. Hypha bearing terminal series of empty antheridia and one empty oogonium. × 330.

Fig. 3. *M. macrandra* var. *macrandra.* Hyphal tips bearing an empty oogonium from which the oospore has fallen away; an oogonium showing a stage in the evacuation of the oospore; a terminal antheridium the contents of which have divided to form antherozooids. × 330.

(*Accepted for publication* 15 *November* 1954)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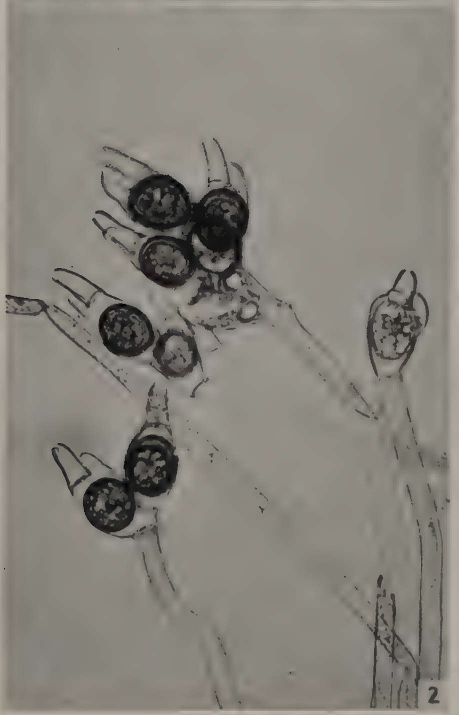
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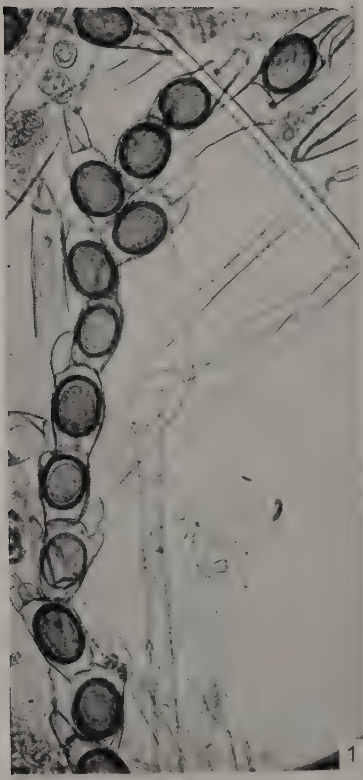

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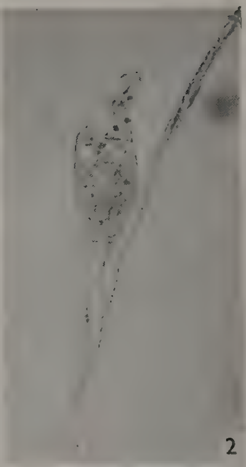

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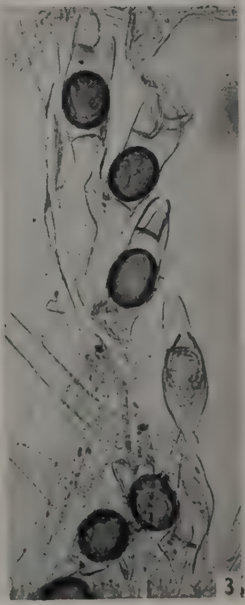

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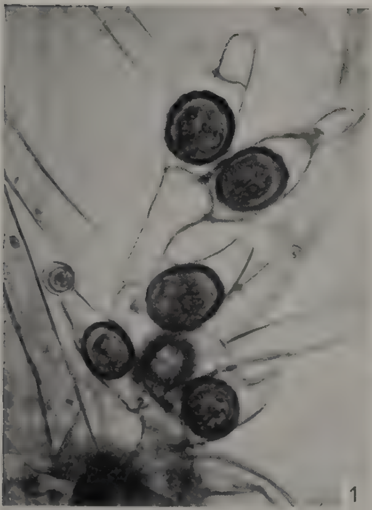

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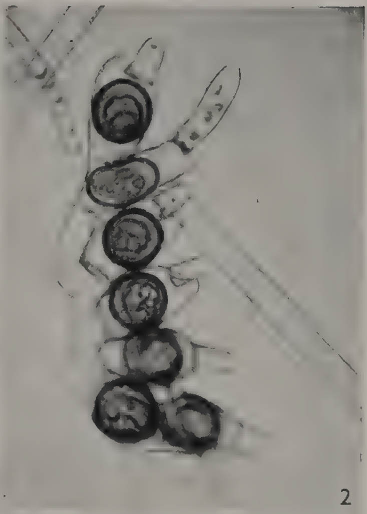

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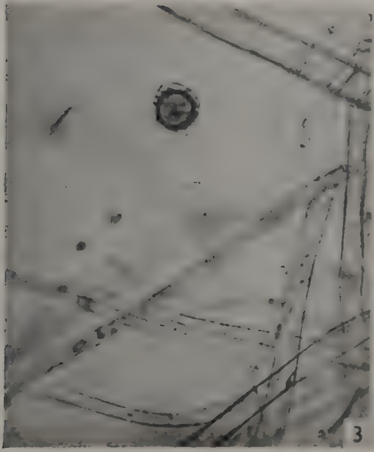

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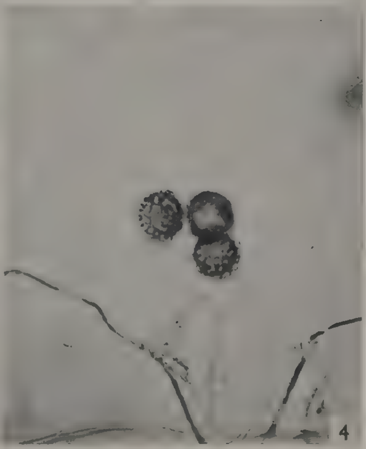

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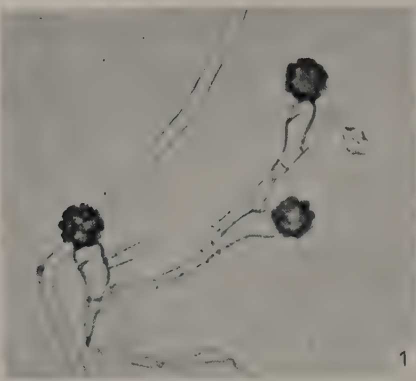
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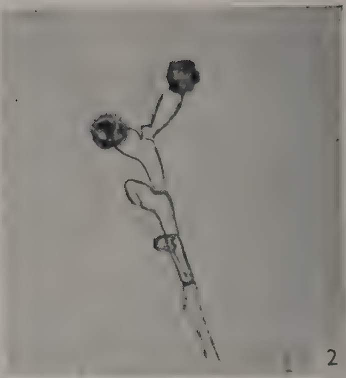
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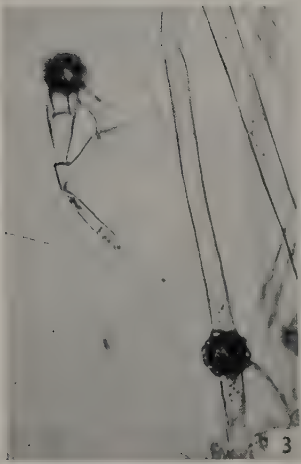
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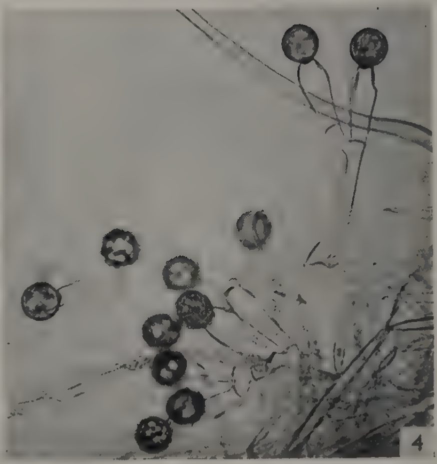
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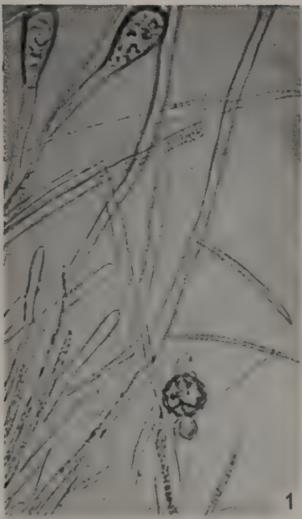
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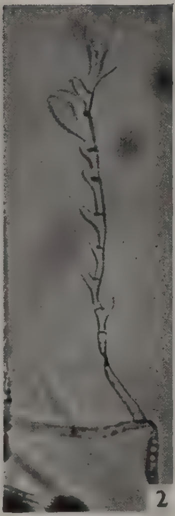
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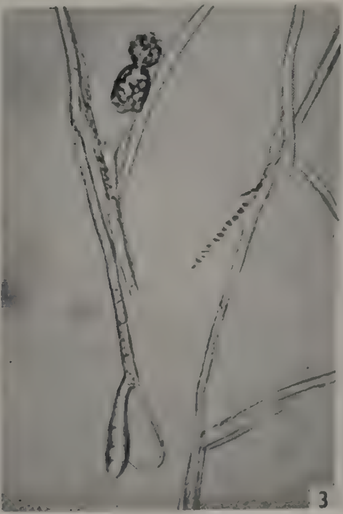
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Trans. Brit. mycol. Soc. **38** (3), 283–290 (1955).

STATISTICAL ASPECTS OF FUNGUS FORAYS

By A. F. PARKER-RHODES

Since a large part of our floristic knowledge of the higher fungi comes from fungus forays, it is desirable to know what sort of efficiency is attained in these records, and how they compare with other possible methods of work. These questions are here examined statistically, with the help of records obtained during the past five years in 'fungus weeks' held at Flatford Mill Field Study Centre in south-east Suffolk.

It is found that the efficiency with which species are recorded is low, the best individual occasions giving about 25% of the total number which exist in the locality. This, however, appears to be due almost entirely to the erratic fruiting of the fungi themselves, and not to the inefficiency of the forayers. As a result, long periods of time are required to get an adequate knowledge of any local mycota. Difficulties likely to be encountered in attempts to apply the methods to extensive series of records are mentioned.

Statisticians may be interested in the method used to estimate total species lists from cumulative records.

The general conclusion is that fungus forays are as good a method of study of floristic questions as can be expected, but would be better if they were smaller and more frequent.

The Fungus Foray is in many ways a unique institution. No other class of botanists is so gregarious or so seasonal, or so prone to combine scientific with social objectives. The origin of this condition is of course in the special habits of the objects of study; but it nevertheless imposes on the serious student certain problems of interpretation which do not occur in other branches of biology. The fact is that quite a large part of our floristic knowledge of the higher fungi in Western Europe is derived from the records of forays, and it is desirable to know, if possible, how far such knowledge can be relied upon. This paper is an attempt to consider this question by statistical methods, applied to actual field records.

The nature of fungus forays

It is obvious that, considered from the standpoint of the theory of experimental design, a fungus foray leaves much to be desired. Two or three dozen people, wandering in a wood, as often as not in single file, or in compact groups round some relative expert, and whimsically collecting specimens in baskets, some because they know what they are and some because they do not, is not very tractable material for the statistician. However, it does result in finding a lot of fungi, and if someone is to hand who can identify them correctly and make a list of them, the records of the outing can ultimately be subjected to rational analysis. Of course, even here there are snags; there is more than one way by which a name can get on to such a list, not to mention the silent mendacity of its omissions. But in the long run such vagaries ought, under proper treatment, to be subsumed within the general variance of whatever conclusions the statistician may draw, unless (as is all too likely in this country) the records are too much dominated by the personal idiosyncrasies of particular individuals.

It would be of interest, perhaps, to apply the methods described in this paper to the accumulated records of the British Mycological Society (or, better still, of the Société Mycologique de France); but it is much easier to start with some more tractable and better controlled material. For this reason I give here an analysis of the results obtained over the past five years during 'fungus weeks' organized at the Flatford Mill field study centre of the Council for the Promotion of Field Studies.

Description of the procedure

These occasions are of the nature of fungus forays, though they differ in certain respects from the type of the species. First, the number of participants is smaller; it has varied from three to thirteen. For the statistician this is an advantage, because one might expect to obtain a better view of the operations of each person than can be had in a field of fifty. Another point in favour of the Flatford forays is that all those taking part have an avowed intention to learn, so that their treatment of the records is a little more reliable than in the average case. But the real point which makes the records susceptible to analysis at all is that the same localities are visited every year; a much longer time span would be needed to obtain a comparable series out of the Society's records, and in that longer span the incidence of ecological changes could not be ignored, as it has been here.

The field procedure is very much like that of any other fungus foray; the morning is spent in searching the chosen locality, with as much dispersion as the terrain permits, and operations are concluded when it appears that the material already collected is as much as can be dealt with before bed-time. This is of course an unsatisfactory stopping criterion, as it tends to mask real differences in the numbers of species fruiting in different places or at different times; but it is imposed by the natural desire of the participants not to waste their time. With a few exceptions all the identifications have been made by myself; while this does not necessarily increase the taxonomic accuracy of the records, it certainly increases their self-consistency, which is again an advantage for statistical purposes. The exceptions were the occasions when we had the benefit of Mr Pearson's attendance; however, it appears that the number of species which would have been differently named in his absence is too small to affect the result appreciably.

As in all fungus forays, no acceptable efforts were made to record numbers of individuals; the only part of the records which can be used is that asserting the presence of a given species on a given occasion. This is of course a severe limitation; but in any case the only information which foray records can ever give is of a floristic nature; that is, that such and such a species does occur, or has occurred, in such and such a locality. However, on one occasion an experiment was made in securing a complete census of all the larger basidiomycetes in a small wood; the results are of some interest, and will be referred to below. But this is not strictly relevant to the subject of the paper, since the procedure is hardly practicable on typical forays.

THE BASIC DATA

The data which form the subject of the analysis are tabulated in Table 1. Here the results of each year are given in successive columns, and those for each locality in successive pairs of rows, the upper row giving the number of species recorded in the given year, and the lower row the number recorded up to that year. It will be seen that the total list up to 1954 contains 575 names. This figure is based on a wide view of the species, and could be appreciably increased on a narrower view. It has been my practice to resolve the contradiction between the splitters and the lumpers by using an unofficial trinomial nomenclature, in which the third epithet may refer to anything from what some would call a mere forma to what others would claim as a full species; in this way the maximum of information is retained in the records with the least multiplication of questionable taxa. In analysis of this kind it is simplest to ignore the trinomes altogether, and this is what I have done in the above figure of 575 species. The taxonomic field comprises the Eubasidiomycetes—that is, all Basidiomycota other than rusts and smuts.

Table 1. *Number of species recorded at seven localities*, 1950–4

Locality		1950	1951	1952	1953	1954
Assington Thicks	Current	17	—	54	72	66
	Cumulant	17	—	61	100	135
Dodnash Wood	Current	47	61	93	97	77
	Cumulant	47	89	139	185	218
Flatford (fields)	Current	49	43	27	29	25
	Cumulant	49	70	82	98	108
Hollesley Heath	Current	50	58	33	48	53
	Cumulant	50	87	102	114	138
Mistley	Current	20	—	34	43	21
	Cumulant	20	—	51	85	94
Stour Wood	Current	25	—	51	55	76
	Cumulant	25	—	70	100	144
Wake-Walker Estate	Current	33	76	85	81	72
	Cumulant	33	86	136	171	192
Total list	Current	207	185	257	265	283
	Cumulant	207	283	389	480	575

CALCULATION OF NUMBER OF SPECIES

For the purpose of assessing the relative significance of these records, it is necessary first to estimate the total number of species which exist, and are susceptible of being recorded at the time of year during which the fungus weeks have been held (late September or early October), in each locality. The way this is done will now be described.

Let p_i be the probability of any species being recorded in the year i. It will depend on two factors, one being the intrinsic probability of observing anything which it is possible to overlook, which for a given species, and the given mode of observation, may be presumed constant; this we shall denote by ϖ. The other factor is the frequency (i.e. proportion) of all the species which are observed in the given time and place. This we shall denote by f_i. Now f_i may be regarded as a 'realization' of ϖ over all the species, and ϖ as a 'generalization' of f_i over all the years. If sampling is unbiased in both respects, p_i will be a symmetrical function of ϖ and f_i. It is also evident that p_i will tend to 1 if either ϖ or f_i tends to 1 (for if the given species is invariably recorded, it will be recorded in the year i; and if every species is recorded in that year, so will this one be); and conversely it will tend to 0 if either ϖ or f_i do so. If we have no knowledge about the species at all, not knowing whether it is an abundant fruiter (for which $p_i > f_i$) or a sparse one (when $p_i < f_i$), we must according to the usual convention put ϖ equal to $\frac{1}{2}$, and in this case we must assume that $p_i = f_i$. The simplest function which meets these requirements is

$$\left.\begin{aligned} p_i &= 1-(1-\varpi)^{F_i}, \\ \text{where} \quad F_i &= \log \frac{1}{1-f_i} \Big/ \log 2. \end{aligned}\right\} \tag{1}$$

If a given species has not been recorded in n successive years, the probability that it exists is given by an estimate of its ϖ which takes due account of this history; $\varpi = 0$ implies that it does not exist. For this purpose, the values of ϖ must be *weighted* by the respective probabilities of the species having escaped observation for each value of ϖ. These probabilities are obviously the corresponding values of

$$P_n = \prod_{i=1}^{n} (1-p_i), \tag{2}$$

which, substituting from (1), becomes

$$P_n = (1-\varpi)^{\sum_{i=1}^{n} F_i}. \tag{3}$$

Therefore, the probability that the species exists, after not having been recorded for n years, is

$$\varpi'_n = \int_0^1 \varpi (1-\varpi)^{\sum_{i=1}^{n} F_i} d\varpi \Big/ \int_0^1 (1-\varpi)^{\sum_{i=1}^{n} F_i} d\varpi, \tag{4}$$

which evaluates as

$$\varpi'_n = 1 \Big/ \left(2 + \sum_{i=1}^{n} F_i\right). \tag{5}$$

If the number of species observed up to the jth year is L_j then the number of new species, observed for the first time in this year, is

$$\Delta L_j = L_j - L_{j-1};$$

and ΔL_j is an estimate of the total number of species remaining unobserved, multiplied by p_j. If the total number of species existing is N, then the *expected* value of ΔL_j will be

$$\Delta\Lambda_j = (N - L_{j-1})\, p_j, \tag{6}$$

but since we already know the history of the first $j-1$ years, *this* p_j depends not on ϖ but on the modified value ϖ'_{j-1}.

Therefore we write, from (1),

$$p_j = 1 - (1 - \varpi'_{j-1})^{F_j}, \tag{7}$$

whence, from (5),

$$p_j = 1 - \left\{ \frac{1 + \sum\limits_{i=1}^{n} F_i}{2 + \sum\limits_{i=1}^{n} F_i} \right\}^{F_j}, \tag{8}$$

so that

$$\Delta\Lambda_j = (N - \Lambda_{j-1}) \left[1 - \left\{ \frac{1 + \sum\limits_{i=1}^{n} F_i}{2 + \sum\limits_{i=1}^{n} F_i} \right\}^{F_j} \right]. \tag{9}$$

This equation enables us to compute the expected curve of growth of the species list in successive years. The expected number of species on the list after t years will be

$$\Lambda_t = \sum_{j=1}^{t} \Delta\Lambda_j = \sum_{j=1}^{t} (N - \Lambda_{j-1}) \left[1 - \left\{ \frac{\log 2 - \sum\limits_{i=1}^{j-1} \log (1 - f_i)}{\log 4 - \sum\limits_{i=1}^{j-1} \log (1 - f_i)} \right\}^{\left(\log \frac{1}{1 - f_j}\right) / \log 2} \right]. \tag{10}$$

In applying this formula, we must observe that $f_i = S_i / N$, where S_i is the number of species actually recorded in the year i, so that Λ_t is a very complicated function of N. Thus the exact estimation of the latter quantity from the given data by any of the standard procedures is very cumbersome. However, the variance of any estimate we may obtain is inevitably rather large, so that it will be sufficient for our purposes to use a graphical method of solution. For this purpose we compute Λ_t from (10) for a series of plausible values of N, and for each year of the records, and calculate the value of χ^2 on comparison of the calculated growth of the list with that observed. The value of N for which χ^2 is a minimum can then be determined graphically, and by the same method fiducial limits of the estimate can be worked out. The resulting estimate is consistent and efficient, though subject to numerical inaccuracy.

TABULATION OF RESULTS

In Table 2 are given the results of this calculation for each of the localities named, together with the consequent figures for the proportion of the species which, thus determined, *can* be found in each locality which actually *were* found in each year. It may reasonably be claimed that these figures are fairly typical of what we may expect from fungus forays generally.

As an indication of the sort of success obtained by the formula (10) in predicting the rate of growth of the species lists, the following may be taken as typical; it refers to the records from the neighbourhood of Flatford:

	1950	1951	1952	1953	1954
Species list observed	49	70	82	98	108
Calculated from (10), assuming $N=200$	49*	74·8	86·1	96·1	103·4

* This figure is of course given by identity.

The fit is reasonably good, with $\chi^2=0{\cdot}76$ among 4 degrees of freedom.

Table 2. *Proportion of species which* were *found to those which* can be *found in the same locality*

	No. of species			Proportion of species recorded in					
Locality	Lower fiducial limit	Estimate	Upper fiducial limit	1950	1951	1952	1953	1954	Proportion recorded up to 1954
Assington Thicks	170	250	400	0·068	—	0·216	0·288	0·264	0·540
Dodnash Wood	280	370	540	0·127	0·165	0·251	0·262	0·208	0·589
Flatford (fields)	130	200	300	0·245	0·215	0·135	0·145	0·125	0·540
Hollesley Heath	170	210	260	0·238	0·276	0·157	0·229	0·252	0·657
Mistley	200	400	700	0·050	—	0·085	0·107	0·052	0·235
Stour Wood	200	370	700	0·068	—	0·138	0·149	0·206	0·389
Wake-Walker Estate	240	330	470	0·100	0·230	0·258	0·245	0·218	0·581
All localities	690	780	930	0·266	0·237	0·330	0·340	0·363	0·737
Minimum no. of participants				6	4	4	3	10	

DISCUSSION

The most striking feature of Table 2 is perhaps the low proportion, of the species which we must believe to be present, which are actually recorded on any one occasion. The highest figures are little over one-quarter. Actually, one may reasonably claim that the true values are lower, since the estimates of the total species list in each locality by their nature exclude any species which are omitted by the chosen sampling methods (for example, those which fruit earlier or later in the year, those which occur in habitats inadequately investigated, or members of groups such as the resupinates which are inadequately represented through limitation of time available for study). The position is much improved when, instead of a single locality, one takes a whole series representing a variety of ecological conditions; in the district studied here one may reasonably expect to record a third of all the species present in any one year (remembering that in each year only 1 week was occupied in the observations). Still more improvement is possible by repeated visits to one locality; after 5 years we have in all cases exceeded 50 % of the estimated total, and in one case have reached this target after only 4 years. By combining both a variety of localities and repeated visitation we have recorded in 5 years nearly three-quarters of all the species.

These figures gain in significance if compared with a comparable set of results obtained by methods different from those of the foray. The results of my investigations on Skokholm Island, expressed as the proportion of the estimated total of species available for observation actually recorded, are as follows:

		Sampling category		
	Total	Open grassland	Under bracken	Lignicolous
After 5 years	0·515	0·635	0·538	0·200
After 7 years	0·690	0·785	0·689	0·470

It is evident from these figures that one person, working alone, is even less efficient than a group of half a dozen people as a means of sampling the fungus flora of a restricted area. This is the more evident, as the Skokholm records represent a considerably longer period per year spent in actual field work. It may perhaps be objected that the ecological conditions obtaining on Skokholm Island are too different from those of the mainly woodland districts visited from Flatford for the results to be comparable. However, the conditions are closely comparable with those in the locality labelled 'Flatford' in the above tables, which consists mainly of grassland, and here a comparable efficiency of sampling has been attained after 5 years, with only about a tenth as much time spent in the observations as was employed on Skokholm. It seems to follow that slow rates of approach to a knowledge of the complete flora are inevitable.

This is borne out to some extent by the experiment referred to earlier, of making a complete census of the larger fungi in a single small wood of about 2·5 acres. Ten persons were engaged on this, each taking a strip about 30 yards wide (the wood was conveniently long and narrow) and collecting or recording every individual specimen encountered. Altogether sixty-one species were found, the estimated total from the cumulative records (not given in the tables) being about 250 (but with a rather large variance); thus the census which almost certainly included nearly every species fruiting at the time, covered about a quarter of the total flora. This figure is comparable with the best figures obtained in any other single visit to a locality, and suggests strongly that it is the irregular fruiting habits of the Basidiomycetes which make their recording so slow a matter, and not, as a casual inspection of a foray in progress would lead one to suppose, the inefficiency with which they are sampled by the observers.

If this is so, one would expect that above a certain minimum the numbers engaged on a foray would not have much effect on the number of species recorded. Inspection of the last two rows of Table 2 seems to confirm this; although the highest overall efficiency was obtained by the largest field, the next best figure (not so very far behind) was that of the smallest.

This prompts the suggestion that if mere information were the main purpose of the affair it would be much more effective to hold many small forays than a few large ones. However, fungus forays are social occasions (because, I suppose, the very erraticness of the behaviour of the fungi lends a quality of fascination to the procedure lacking from most biological observations), and the suggestion can hardly be taken very seriously.

Under present conditions it would take about eighteen years for the Flatford fungus weeks to record 99 % of their available species, and nearly thirty years for me to attain that standard on Skokholm. In a territory the size of Britain (which is very large compared to its population of mycologists) we are probably still far short of this figure after about 100 years. There is, however, a complication, in applying such results as these to a wider field, whether of space or of time, and that is that different authorities recognize different species. By the use of a new system of classification in which new or different 'species' are recognized, a large apparent addition to any list can be made with no trouble at all. This must have happened many times since foray records were first compiled, and (until a stable convention of classification is agreed upon) will go on happening, and for this reason it is doubtful whether a statistical study of these records would at present have much meaning.

(*Accepted for publication* 14 *December* 1954)

Trans. Brit. mycol. Soc. **38** (3), 291–297 (1955).

EFFECTS OF OXYGEN-NITROGEN MIXTURES ON THE SPORE GERMINATION OF MUCORACEOUS MOULDS

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Using species of Mucorales, spore germination has been studied in relation to oxygen concentration. With *Mucor hiemalis*, *Phycomyces blakesleeanus* and *Rhizopus stolonifer*, all weak fermenters, there is no germination in the absence of oxygen. Small increases of percentage oxygen in the gas stream circulating through the spore suspension lead to increased germination. In *Mucor racemosus* and *M. rouxianus* there is much budding and some germination in the absence of oxygen, and the percentage germination increases as the oxygen concentration is increased. The degree of germination in oxygen as compared with that in air varies with the species and with the temperature employed.

There are two stages in the germination of a spore: swelling and germ-tube production. Oxygen is essential only in the second stage.

Introduction

Much of the literature concerning the effects of oxygen on spore germination has been summarized by Gottlieb (1950) who remarks: 'while oxygen may be indispensable for the germination of fungus spores, comparatively few exacting experiments to support this concept have been reported, and most evidence is of indirect observations'. The work presented here is an attempt to place on a more exact basis the effect of oxygen concentration on germination.

Germination was assessed in a liquid medium, agitated by a suitable gas stream.

Cultures of the fungus to be tested were grown on test-tube slopes of 2 % malt agar, except for *M. rouxianus* which will not sporulate easily on this medium and was therefore grown on oatmeal agar. The incubation period and temperature for any one species was the same in all experiments, but each species had to receive individual treatment, having a rate of germination and temperature optimum differing from the other species. In *M. hiemalis* the (−) strain was used throughout, although it has been found that the (+) strain reacts in a similar manner.

A spore suspension was normally prepared by washing off the spores with a sterile 2 % malt extract solution, 100 ml. being used to wash spores from two test-tube slopes. 20 ml. of this suspension were transferred to a sterilized boiling tube fitted with a 'Quickfit' joint and gas-head; the long arm of the gas-head being drawn out to a fine point to reduce the size of the entering bubbles. Care was taken when using the malt extract solution not to include any of the precipitate formed on autoclaving, as spores tend to become entangled in the flocculant thus impeding attempts to keep spores equally spaced in the solution. Malt-extract solution was shown to give much better results as a germinating medium than any synthetic nutrient solution tried.

Tubes were connected together in series with pressure tubing, and immersed in a thermostatically controlled water-bath. On the basis of preliminary experiments the duration of the experiment was arranged to give 80–90 % germination at the optimum temperature for the fungus with air as the gas stream.

The gas mixtures provided by the British Oxygen Company,* were used without previous treatment. Nitrogen was first bubbled through alkaline pyrogallol to remove any traces of oxygen. When air was used it was drawn through by means of a constant level aspirator, after preliminary scrubbing with caustic soda to remove any carbon dioxide. Efforts were made to see that the rate of bubbling was approximately constant in each experiment, although it has been shown experimentally that the rate of bubbling does not affect the germination of spores, within very wide limits.

At the end of a suitable period the spores were preserved by adding iodine before counting.

The average percentage germination was calculated from 10 counts of 100 spores each, any spore whose germ tube was as long or longer than the diameter of the spore was counted as having germinated. This method is a compromise between the two measures considered by Tomkins (1932) to give a true picture of the amount of germination, namely the percentage of spores germinated and the average length of the germ tube.

RESULTS

(1) *The effects of oxygen-nitrogen mixtures on the germination of spores of* Mucor hiemalis, M. racemosus, M. rouxianus, Phycomyces blakesleeanus *and* Rhizopus stolonifer *at various temperatures* (*Tables* 1–6)

In spite of all precautions (using cultures of the same age, spore suspensions of the same approximate density, media of the same concentration and similar rates of bubbling) it was not found possible in any two experiments to obtain exactly similar results, although there was normally general agreement between two similar experiments (Table 3).

One fact stands out clearly: those fungi (*Mucor racemosus*, *M. rouxianus*) which are known to produce alcohol readily will germinate in the almost complete absence of oxygen. *M. hiemalis*, known to be a weak fermenter, will not germinate in malt solution in equilibrium with 'cylinder' nitrogen after 7·5 hr., but after 2 days some spores do germinate. *Phycomyces blakesleeanus* and *Rhizopus stolonifer* known to be extremely poor alcohol producers, will germinate only if appreciable oxygen is present.

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* The purities of the samples are quoted as follows:

(1) *Pure nitrogen*
Nitrogen – above 99·9 %
Oxygen < 10 vol. per million
Main impurities He and Ne

(2) *Commercial nitrogen*
Nitrogen ≏ 99·9 %
Oxygen – 0·05 %

(3) 2 % *oxygen* : 98 % *nitrogen*
Made up from (2) and (6) with tolerance ± 0·1 %

(4) 5 % *oxygen* : 95 % *nitrogen*
Made up from (2) and (6) with tolerance ± 0·5 %

(5) 50 % *oxygen* : 50 % *nitrogen*
Made up from (2) and (6) with tolerance ± 2·0 %

(6) *Commercial oxygen*
Over 99·5 % oxygen. Impurities, argon and nitrogen.

Table 1. *Spore germination in* Mucor racemosus *Fresen.*

Cultures, used for making spore suspensions, grown on 2% malt extract agar for 7 days at 20° C. before use. Gas bubbled through spore suspension for 7·5 hr.

Temperature (° C.)	Oxygen (%)	Germination (%)	Standard deviation
25	0	8·9	3·8
	2	20·0	1·05
	5	29·4	4·31
	20 (air)	41·7	4·87
	100	61·0	7·75
30	0	3·4	1·7
	2	21·2	5·35
	5	35·4	6·22
	20 (air)	70·9	3·87
	100	89·9	3·82

Table 2. *Spore germination in* Mucor rouxianus (*Calmette*) *Wehmer*

Cultures used for making spore suspension grown on oatmeal agar for 5 days at 30° C. before use. Gas bubbled through spore suspension for 6 hr.

Temperature (° C.)	Oxygen (%)	Germination (%)	Standard deviation
(1) 30	0	38·4	4·35
	2	65·2	7·93
	5	79·2	5·63
	20 (air)	74·9	6·40
	50	75·4	6·08
	100	98·2	0·92
(2) 38·5	0	62·2	8·52
	2	56·3	5·58
	5	62·5	3·63
	20 (air)	91·2	2·97
	50	93·8	2·44
	100	92·6	2·95

Note. At 40° C. the spores swell but do not produce germ tubes in a period of 6 hr.

That any of these fungi are capable of strictly anaerobic germination is possibly open to doubt. Using methylene blue as an indicator in the nutrient solution it takes 10–30 min., depending on the rate of bubbling, for a solution agitated by a stream of pure nitrogen to become completely free from oxygen. Thus in the early stages of an experiment conditions are not quite anaerobic. True anaerobic conditions are very difficult to attain, but when obtained *Mucor rouxianus* and *M. racemosus* respond by budding and by abnormal germ-tube production similar to that observed by Duckworth & Harris (1950) in *Penicillium chrysogenum*. Where anaerobism is incomplete, the minute amount of dissolved oxygen may be sufficient to stimulate normal germ-tube production.

Table 3. *Spore germination in* Mucor hiemalis *Wehmer*

Cultures used for making spore suspensions grown on 2% malt-extract agar at 20° C. for 7 days before use. Gas bubbled through for 7·5 hr. For both temperatures A and B are separate experiments.

Temperature (° C.)	Experiment	Oxygen (%)	Germination (%)	Standard deviation
25	A	0	0	0
		2	27·7	6·68
		5	44·6	2·79
		20 (air)	86·4	2·41
		50	—	—
		100	85·1	4·15
	B	0	0	0
		2	2·3	1·06
		5	8·6	1·39
		20 (air)	48·5	5·29
		50	51·5	5·55
		100	71·1	3·65
30	A	0	0	0
		2	29·7	3·49
		5	54·4	6·79
		20 (air)	91·8	1·64
		50	—	—
		100	45·1	8·25
	B	0	0	0
		2	7·9	3·36
		5	—	—
		20 (air)	32·3	5·33
		50	37·8	6·78
		100	32·5	3·34

Table 4. *Spore germination in* Phycomyces blakesleeanus *Burgeff*

Cultures used for making spore suspensions grown on 2% malt-extract agar for 20 days at 20° C. before use. Gas bubbled through for 24 hr.

Temperature (° C.)	Oxygen (%)	Germination (%)	Standard deviation
20 (near growth optimum)	0	0	0
	2	27·8	1·92
	5	29·2	1·65
	20 (air)	43·4	3·58
	100	52·2	1·49
25 (just above growth optimum)	0	0	0
	2	14·3	3·77
	5	17·6	5·74
	20 (air)	32·0	6·93
	50	27·5	3·95
	100	29·9	3·63

At 30° C. the spores had not germinated after 24 hr. in the malt solution with air bubbling through.

The effects on germination of increasing oxygen concentration above that of air are somewhat conflicting. Conflicting results are also reported in the literature. Brown (1922) found that oxygen concentration over 80% retarded the germination of *Botrytis cinerea* spores. Child (1929), however, reported that the spores of *Daldinia concentrica* will germinate better in oxygen than controls grown in air; although under similar conditions *D. vernicosa* shows no difference. Wilkins (1938) doubted if Child's experiments were done on a sufficiently large scale to have statistical significance.

Table 5. *Spore germination in* Rhizopus stolonifer (*Ehrenb. ex Fr.*) *Lind.* (*Syn.* R. nigricans *Ehrenb.*)

Seven-day culture grown on malt-extract agar at 20° C. used for spore suspension. Gas bubbled through spore suspension for 7·5 hr.

Temperature (° C.)	Oxygen (%)	Germination (%)	Standard deviation
20	0	0	—
	2	47·6	3·5
	5	54·8	2·7
	20 (air)	65·6	5·5
	50	71·2	6·2
	100	69·2	5·4
25	0	0	—
	2	24·3	2·0
	5	58·7	7·1
	20 (air)	96·9	1·7
	100	95·8	2·0

The effects of increasing the oxygen concentration on spore germination in the present experiments (Table 6) show that, in *Mucor racemosus* and *M. rouxianus*, the percentage germination in pure oxygen is significantly higher than that in air at the temperatures tested. For *M. hiemalis* in one experiment at 25° C. there was no significant increase of percentage germination in oxygen as compared with air, whilst at 30° C., one experiment indicated a significant decrease, although in another this change was not significant. *Rhizopus stolonifer* shows no significant difference at either temperature, whilst *Phycomyces blakesleeanus* shows a significant increase only at 20° C.

Table 6. *Effect of oxygen on spore germination*

Species	Temperature (° C.)	Mean % germination in air	Mean % germination in oxygen	Value of *P* in comparison of two means	*
Mucor racemosus	25	41·7	61·0	< 0·001	+
	30	70·0	89·9	< 0·001	+
M. rouxianus	30	74·9	98·2	< 0·001	+
	38·5	91·2	92·6	0·02	+
M. hiemalis	25	86·4	85·1	0·9	o
	25	48·5	71·1	< 0·001	+
	30	91·8	45·1	< 0·001	–
	30	32·3	32·5	0·9	o
Rhizopus stolonifer	20	65·6	69·2	0·3–0·5	o
	25	96·9	95·8	0·1–0·2	o
Phycomyces blakesleeanus	20	43·4	52·2	< 0·001	+
	25	32·0	29·9	0·2–0·3	o

* Significant increase, +; significant decrease, –; difference non-significant, o.

The general picture seems to be that those species capable of germination at very low oxygen tensions are more sensitive to increases in oxygen concentration than those species which are more markedly aerobic.

(2) *Oxygen requirements for first stage (swelling) and second stage (germ-tube production) of germination*

Four tubes (*A*, *B*, *C* and *D*) of *Mucor hiemalis* spores suspended in 2 % malt extract solution were kept at 25° C. for 8 hr. In *A* air was bubbled through for the whole 8 hr. and through *C* nitrogen was passed. Through *B* air was bubbled for the first 4 hr. and nitrogen for the second 4 hr.; *D* receiving the reverse treatment. At the end of the experiment the percentage germination was determined for each tube in the usual way. The results are given in Table 7. In two similar tests (Table 8) with *M. rouxianus* at 37·5° C. the period of experiment was 6 hr.

Table 7. *Oxygen requirements for the first and second stages of germination of* Mucor hiemalis

	Treatment		Average % germination	Standard deviation
	First 4 hr.	Second 4 hr.		
A	Air	Air	70·3	11·8
B	Air	Nitrogen	8·6	3·6
C	Nitrogen	Nitrogen	0	0
D	Nitrogen	Air	60·4	12·38

Table 8. *Oxygen requirements for the first and second stages of germination of* Mucor rouxianus

	Treatment		Average % germination	Standard deviation
	First 3 hr.	Second 3 hr.		
A	Air	Air	65·8	8·2
B	Air	Nitrogen	12·8	4·3
C	Nitrogen	Nitrogen	10·9	3·8
D	Nitrogen	Air	81·4	5·9
A	Air	Air	72·7	6·2
B	Air	Nitrogen	27·4	5·0
C	Nitrogen	Nitrogen	12·6	2·9
D	Nitrogen	Air	77·2	6·2

These experiments would appear to show that the first stages of germination, when the spore is simply swelling, oxygen is of no significance, and it is only in the second stage, when germ tubes develop, that it assumes importance.

I wish to thank Professor C. T. Ingold for his unfailing help and encouragement.

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(*Accepted for publication 4 January* 1955)

Trans. Brit. mycol. Soc. **38** (3), 298–301 (1955).

ON THE ORIGIN OF COLONIES OF FUNGI DEVELOPING ON SOIL DILUTION PLATES

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A method has been devised for determining the nature of the propagules (i.e. spores or hyphae) that give rise to individual colonies of fungi on soil dilution plates. Soil dilution plates of clear, filtered Dox + yeast agar (pH 4·2–4·4) are incubated for 18 hr. at 25° C., and are then searched for young fungal colonies, each of which is removed in a small agar block for direct microscopic examination. After the nature of the fungal propagule giving rise to the young colony has been determined, the agar block is transferred to fresh medium, to permit growth and identification of the fungus. Using this technique, the majority of the colonies developing on soil dilution plates were found to have arisen from spores.

The dilution-plate method for the study of soil fungi has been adapted by soil mycologists from the method originally developed for the isolation and study of soil bacteria (Waksman, 1927). When this method is applied, however, to the 'counting' of soil fungi a serious difficulty arises owing to the fact that any given fungal colony on a plate might have originated from a fragment of active mycelium or from an inactive spore. Since many of the abundant fungi, *Mucor*, *Aspergillus*, *Penicillium*, *Trichoderma* etc., isolated by the dilution-plate method are species that spore heavily, it is generally considered that a major defect of the method is the substantial advantage it gives to heavily sporing fungi (Garrett, 1951). As far as the writer is aware, however, there are no published data on what proportion of the colonies developing on dilution plates may arise from spores; further, the view that most colonies on such plates arise from spores has recently been questioned (McLennan & Ducker, 1954).

During a survey of the fungi occurring in a wheat-field soil at the Waite Institute, Adelaide, South Australia, the origin of fungal colonies developing on dilution plates has been studied. The method has been to search dilution plates for young developing colonies, to remove each colony in a small agar block for direct microscopic examination to determine the unit of origin of the colony, and to transfer each block to fresh medium for further growth and subsequent identification of the fungus.

Dilution plates of surface soil (1 in. depth) were prepared at a dilution of 1:5000, the medium being acid Dox + yeast agar (pH 4·2–4·4) (Warcup, 1950). To facilitate the search for young fungal colonies the medium was filtered to remove all suspended particles; likewise the Petri plates were washed and carefully dried free of traces of lint. After incubation for 18 hr. at 25° C. the plates were inverted under a dissecting microscope (× 12·5) and examined for the presence of developing fungal colonies; all colonies seen were ringed with a china-marking pencil. Plates were then reversed and each colony cut out in a small block of agar, placed on a sterile slide and examined under a high-power microscope (× 120 and × 540) to trace the origin of the colony. The agar block was then placed on the surface of a fresh plate of Dox + yeast agar, incubated, and the fungus allowed to develop for identification. Plates were examined twice daily for removal of fungi until no further colonies could be isolated, a period of 6–14 days. After the initial incubation at 25° C. plates were kept overnight at 12–15° C. to prevent rapid growth of fungi during that period, and at room temperature during the day. If a colony of any fungus that formed a dense, copiously branched growth was overlooked, at the next examination it sometimes proved impossible to discern the origin of that colony. After the initial opening of a plate any colony developing on the surface of the agar was discarded as a probable aerial contaminant.

The origin of fungal colonies developing on three dilution plates prepared from each of eleven different samples of wheat-field soil are recorded in Table 1. The units of origin are given as 'spores', 'hyphae', 'humus particles', and 'unclassified'. Those colonies where the growth arose from a black or brown particle are classed as 'humus particle', such a group covering both spores or hyphae embedded in, or attached to, humus particles. By squashing a 'humus particle' under a coverslip it was often possible to see whether the growth originated from spore or hypha; however, since such colonies were killed and could not be further identified, this method was used only in preliminary studies. Unclassified colonies were usually cases where the colonies had grown so profusely that it was impossible to discern the origin of the growth; a few cases were noted, however, where, although the unit of origin was seen, it was impossible to decide its morphology. Table 1 shows that on all eleven series of dilution plates the majority of the colonies recorded originated from spores and that hyphae were rare, even though many of the soil samples were collected during the winter and spring, a period when soil moisture is not a limiting factor for plant or microbial growth at Adelaide.

Table 1. *Numbers of fungal colonies developing from spores, hyphae or humus particles on dilution plates prepared from eleven samples of wheat-field soil*

		No. of colonies developing from			
Sample no.	Moisture content (% dry wt.)	Spores	Hyphae	Humus particles	Unclassified
15	23·0	78	6	16	12
16	21·7	60	6	17	5
17	21·3	71	6	7	0
18	18·9	58	9	32	4
19	18·3	52	8	12	4
20	18·5	82	4	18	4
21	12·4	205	0	28	14
22	9·6	50	1	23	4
23	6·8	45	1	25	2
24	3·5	33	0	13	2
25	11·7	37	0	15	4
	Totals	771	41	206	55
	Percentage	70	4	19	5

The total number of colonies of each species of fungus isolated from spores, hyphae, or humus particles from all eleven soil samples is recorded in Table 2. This table shows that fungi developing from spores comprise the largest group both in number of species and in number of colonies. The Phycomycetes, species of *Absidia*, *Cunninghamella*, *Mortierella*, *Mucor* and *Rhizopus*, were present predominantly as spores and were not recorded

Table 2. *Total number of colonies of each fungus developing from spores, hyphae, or humus particles on dilution plates of wheat-field soil*

	No. of colonies developing from			
Fungus	Spores	Hyphae	Humus particles	Unclassified
Absidia butleri Lendner	4	—	2	—
A. ? ramosa (Lindt) Lendner	—	—	2	—
Cunninghamella elegans Lendner	2	—	—	—
Mortierella: 4 species	10	—	3	2
Mucor: 2 species	8	—	—	—
Rhizopus arrhizus Fischer	36	—	—	—
Chaetomium: 3 species	11	—	3	2
Alternaria tenuis auct.	—	—	1	—
Aspergillus flavus Link	4	—	—	—
A. fumigatus Fres.	—	—	1	—
A. terreus Thom	3	—	—	—
Cladosporium herbarum Link	8	—	2	—
Fusarium oxysporum sensu Snyder & Hansen	13	—	21	—
Fusarium: 2 species	—	—	2	1
Gliocladium roseum (series)	2	—	—	—
Humicola W 61	1	—	4	—
Papularia sphaerosperma (Pers.) von Höhnel	6	—	1	—
Penicillium lilacinum Thom	12	—	5	5
P. melinii Thom	11	—	1	1
P. purpurogenum Stoll	40	—	4	4
P. urticae Bainier	201	—	21	11
Penicillium W 2	188	—	31	17
Penicillium W 3	20	—	7	3
Penicillium: 6 species	26	—	5	5
Pestalotia W 67	2	—	—	—
Phoma: 3 species	4	—	2	—
Pullularia pullulans (de Bary & Low) Berkhout	—	—	4	—
(*Scopulariopsis*?) W 21	106	—	19	3
Stemphylium botryosum Wallr.	8	—	2	—
Stemphylium W 110	3	—	—	—
Thielavia, n.sp.:				
Trichoderma viride Pers. ex Fr.	11	—	1	—
W 118	14	2	2	—
W 82	9	—	8	—
W 10 (sterile culture)	—	—	7	—
T 90	—	—	7	—
T 48 (sclerotia)	—	1	3	—
W 85 (sterile culture)	—	1	2	—
T 60 (sterile culture)	—	5	3	—
T 58 (sterile culture)	—	6	6	—
T 7 (sterile culture)	—	6	4	—
T 49 (sterile culture)	—	12	—	—
T 82 (sterile culture)	—	3	—	—
T 42 (sterile culture)	—	2	—	—
Unidentified rarer fungi: 23 species	8	3	20	1
Total	771	41	206	55

from hyphae. All spores were sporangiospores save for conidia of *Cunninghamella* and two stylospores of *Mortierella*. *Chaetomium* was recorded mainly from spores and occasionally from humus particles, the spores being brown ascospores. Species of *Aspergillus*, *Penicillium*, *Cladosporium* and *Trichoderma* were also present predominantly as spores and occasionally in humus particles. *Fusarium* was one of the small group of fungi isolated more frequently from humus particles than from spores; it also differed from most other fungi occurring as spores in that the spores were chlamydospores and not conidia. The small group of twelve species recorded developing from hyphae form a contrasting group to the species isolated from spores. Only one species, an unidentified Hyphomycete, was recorded both from spores and from hyphae. The identity of the remaining eleven species is not known, for although most have been in culture for several months, not one has yet produced any reproductive structures other than sclerotia; this behaviour being markedly different from the fungi recorded predominantly from spores, most of which form spores readily in culture. Several of the fungi isolated from hyphae were also very slow-growing, often not being noticed on the dilution plates until the third or fourth day after their preparation. Such slow-growing colonies are undoubtedly easily lost under faster-growing species on normal dilution plates where all fungi are allowed to develop on the plate. It is also interesting to note that many hyphae seen on the plates did not grow in the agar medium; some had lost their cell contents, and with others it was impossible to decide if they were dead or alive. Only one non-viable spore, a beaked *Alternaria*-type spore, was seen; others may have been present but would be very difficult to see owing to their small size.

This study has shown that the majority of the colonies isolated from these dilution plates originated from spores present in the soil. As Garrett (1952) has rightly pointed out, however, fungi may exist in soil both as active mycelium and as dormant spores; active vegetative mycelium developing upon freshly exposed substrates, and later giving rise to spores. Although many fungi were recorded here developing only or predominantly from spores, this does not necessarily imply that they are incapable of active growth in soil; they may be active under certain limited conditions or the number of spores of these fungi present in the soil may be much greater than the amount of active mycelium. Several of the Phycomycetes present in this wheat-field soil have, by other methods, been shown to have a short period of profuse active growth in the soil (Warcup, unpublished data).

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(*Accepted for publication* 22 *January* 1955)

Trans. Brit. mycol. Soc. **38** (3), 302–304 (1955).

BRITISH RECORDS, 7–11

7. **Ascozonus woolhopensis** (B. & Br.) Renny, *Trans. Woolhope Nat. Fld Cl.* 1871–73, pp. 127–131, 1873 (*Ryparobius woolhopensis* B. & Br., *Ann. Mag. nat. Hist.*, Ser. 4, **11**, 339–349, 1873, from bird's dung, leg. Renny).

In March 1933, a species of *Ascozonus* was found on rabbit pellets from Tadworth, Surrey. In November 1953, what appeared to be the same species was found on deer and rabbit droppings from Epping Forest. It was isolated in pure culture on dung agar and was also found to grow well on oatmeal agar. Growth occurred on malt agar, but on this medium apothecia were rarely produced.

On agar the mycelium is almost entirely submerged, the only aerial structures being the apothecia which, under favourable conditions, may be produced within a week in a culture started from spores. Light seems to be important for the production of apothecia; in darkness these are rarely formed.

Ascozonus Renny is characterized by the internal ring of thickening of the wall a short distance from apex of the ascus (Fig. 1 B, C). The various species differ from one another in the typical number of spores in the ascus, in spore size, and in the shape of the apothecium (Renny, 1873).

In the fungus under consideration the asci appear to have an ideal spore number of 64, although this total is rarely realized due, no doubt, to the abortion of a few spores. The spores are discharged as a single mass to a height of several centimetres. Measurements of 100 freshly liberated spores, from apothecia grown in pure culture, were $8–17 \times 3–6\,\mu$ (average $12{\cdot}9 \times 4{\cdot}3\,\mu$). In the early descriptions the spore measurements were given as $0{\cdot}0007 \times 0{\cdot}0003$ in. $(= 17{\cdot}8 \times 7{\cdot}6\,\mu)$. This spore size is rather large for the present material. No recognizable type material is available for comparison. Seaver (*N. Amer. Cup-fungi* (*Operculates*), 1928), who places the species in *Streptotheca*, gives a spore size of $12–14 \times 6–7\,\mu$, based on an American collection.

The cream-coloured apothecia are minute, $150–400\,\mu$ across and about $250\,\mu$ high. The form is striking, resembling the capitulum of a spear-thistle (Fig. 1 A). The surface cells of the lower region are extremely large, and in the upper part the excipulum is clothed by numerous short unicellular hairs (Fig. 1 D).

There is little doubt about the identification of this fungus which does not seem to have been reported from Britain since its original discovery.

Material has been deposited in Herb. Kew., and cultures have been sent to the C.M.I., Kew.

WINIFRED M. PAGE, Birkbeck College, University of London

8. ****Trichoglossum walteri** (Berk.) Durand, *Ann. mycol., Berl.*, **6**, 440, 1908. Thirty to forty specimens in sandy lawn, Congleton, Cheshire, October and November 1953 and 1954, accompanied, in the latter year, by plentiful *Geoglossum fallax* Durand (confirmed by Dr J. A. Nannfeldt).

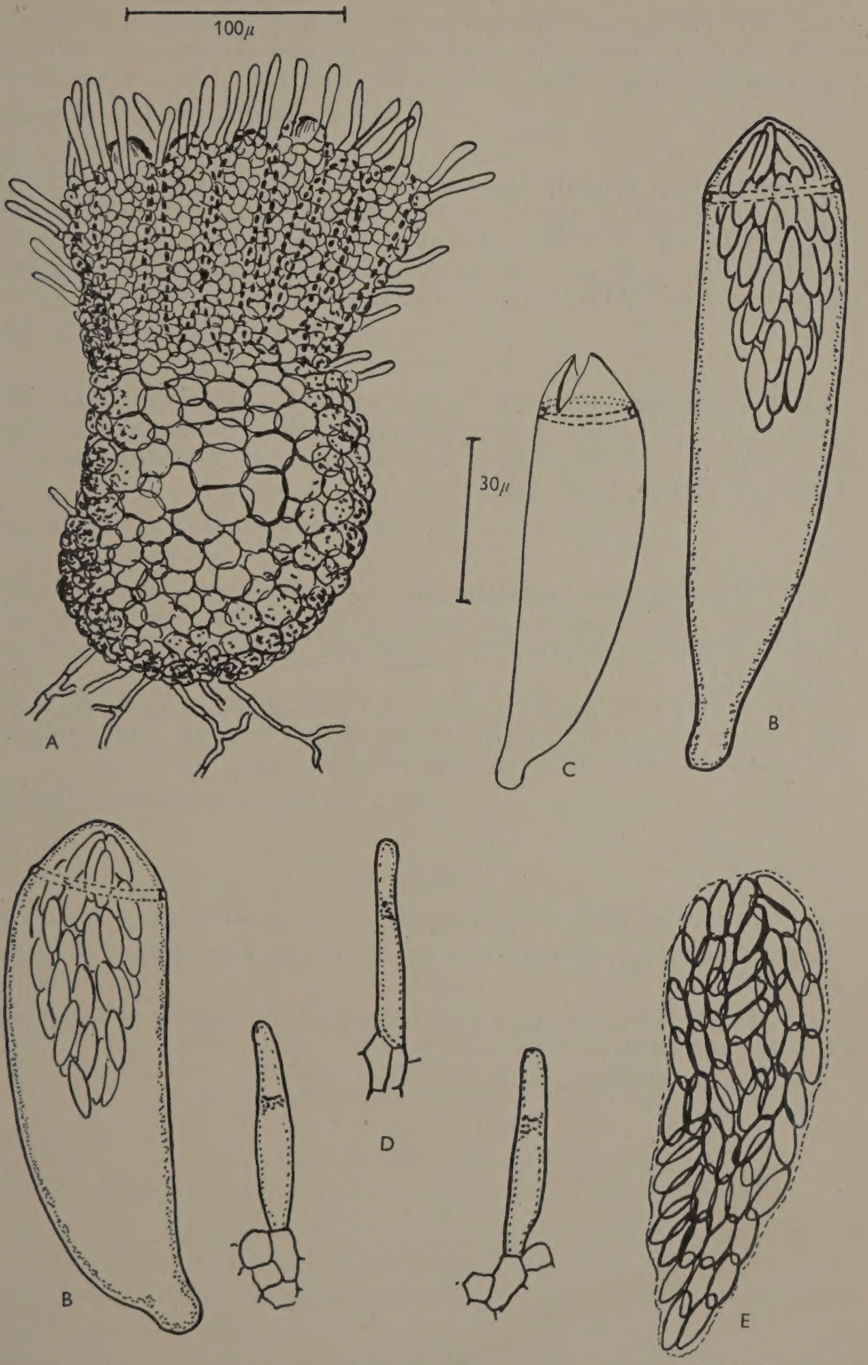

Fig. 1. *Asconzonus woolhopensis*. A, apothecium from pure culture (the outlines of asci seen through the thin excipulum are shown with dotted outlines); B, mature asci; C, ascus after discharge; D, hairs from excipulum; E, discharged spore-mass. (Drawn by C. T. Ingold.)

Much like *Trichoglossum hirsutum* in stature but differing in its shorter spores, 80–105μ, which are constantly 7-septate. There have been few European gatherings. Herb. W.D.G. no. 814, Herb. Uppsala and Herb. Kew.

W. D. GRADDON, Congleton, Cheshire

9. **Hyalotricha crispula** (Karst.) Dennis, *Mycol. Pap. C.M.I.* no. 32, p. 77, 1949. In small colonies on dead stems of *Myrrhis odorata*, Rievaulx Bridge, Helmsley, Yorkshire, June 1954, leg. W. G. Bramley, det. W. D. Graddon. The colonies were growing amongst masses of *Dasyscypha leucophaea*. Herb. W.D.G. no. 920 and Herb. Kew.

W. D. GRADDON, Congleton, Cheshire

10. **Pyrenopeziza thalictri** (Peck) Sacc. (Graddon in *Trans. Brit. mycol. Soc.* **37**, 58, 1954). A second British locality was established when Dr R. W. G. Dennis collected fine material on *Thalictrum flavum* at Wheatfen, Norfolk, June 1954. Herb. W.D.G. no. 907.

W. D. GRADDON, Congleton, Cheshire

11. ****Pyrenopeziza umbellatorum** (Ces.) Rehm, *Ber. bayer. bot. Ges.* **13**, 175, 1912. Good colonies on dead stem of *Heracleum*, Bosley Reservoir, Cheshire, July 1953, det. Dr R. W. G. Dennis. Herb. W.D.G. no. 768 and Herb. Kew.

Apparently a rare fungus of stems of Umbelliferae. Should be rather easily recognized in the field by its flat, bay-brown disk, semi-immersed in the host somewhat in the manner of a *Phacidium*.

W. D. GRADDON, Congleton, Cheshire

CORRECTION

These *Transactions*, **38** (2), 169, 1955, lines 4–6 should read:

J. A. TOMLINSON. Observations on the Crook Root disease of water-cress.

A. P. KOLE. Observations on the behaviour of zoospores of *Spongospora subterranea* and *Olpidium brassicae*.

See *Tijdschr. PlZiekt.* **60**, 1–65, 135–137, 1954.

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Printed in Great Britain at the University Press, Cambridge (Brooke Crutchley, University Printer)